THE POTENTIAL IMPACT OF COCAINE-ASSOCIATED IMMUNE SUPPRESSION ON HUMAN PAPILLOMAVIRUS-ASSOCIATED CLINICAL OUTCOMES

by
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Abstract

Cocaine use has been linked to an increased risk of human papillomavirus (HPV) infection and cervical cancer among women, as well as immune suppressive effects in laboratory and animal settings. We hypothesized that cocaine may impact immune control and reduce the host's ability to suppress HPV replication. However, the role of cocaine use on immunity has not yet been examined in drug-using populations at high risk for HPV infection and cervical cancer. Understanding the natural history of HPV infection in the context of potential cocaine-associated immune suppression may influence clinical management of drug-using women at risk for cervical cancer.

Using human immunodeficiency virus (HIV)-infected and at-risk uninfected Women's Interagency HIV Study (WIHS) participants followed prospectively prior to the widespread use of highly active antiretroviral therapy (HAART), we found nearly half exhibited a diminished epithelial immune response detected using cutaneous anergy testing. We observed a positive association between cocaine use and epithelial immune suppression independent of other risk factors among HIV-uninfected women. In contrast, we were unable to detect a cocaine effect on this immune-mediated response among women with immune-suppressive HIV infection.

We additionally examined the association between cocaine use and incident cervical abnormalities detected by Papanicolau (Pap) smear testing among current and former injection drug-using women in the AIDS Linked to the Intravenous Experience (ALIVE) study. Stratifying our analyses by HIV status, we observed a non-statistically significant increased risk of incident abnormalities among HIV-uninfected women who recently used cocaine. Similar to the cocaine-anergy analysis, we also found no

association among HIV-infected women between cocaine use and abnormal Pap results. However, cervical abnormalities detected by Pap smear may reflect cellular changes not associated with risk of HPV-associated cervical cancer, warranting closer examination. We further examined the effect of cocaine use on HPV-specific cervical cytological abnormalities in a subset of women with HPV genotype data available, but were unable to detect statistically significant risk associations.

Our findings among immune competent women indicate a potential association between cocaine use and dampened cell-mediated immunity at the epithelial cell level. Cocaine-mediated immune suppression may have direct implications for HPV-related risks of cervical abnormalities and cancer.

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Chapter One

Introduction

Human Papillomavirus (HPV) Infection and Cervical Cancer

Papillomaviruses are small, double-stranded DNA viruses that encode only 8 or 9 proteins. They have been in existence for 300 million years (1), and have evolved with their vertebrate hosts (1-4). Papillomaviruses are species-specific viruses, meaning that human papillomavirus (HPV) only infects humans, and also exhibit tissue tropism to squamous epithelia (5).

More than 150 human papillomavirus genotypes have been identified, and they are classified by their epithelial tropisms. Beta, gamma, mu, and nu papillomaviruses infect cutaneous epithelial surfaces, but typically do not cause apparent infections in immunocompetent hosts. Alpha papillomaviruses can infect both mucosal and cutaneous epithelial surfaces. The viral life cycle of HPV is exclusively intraepithelial (6) and does not develop into a systemic infection. It also does not induce viremia or clinically evident inflammation upon initial infection (7, 8). Low-risk mucosal types are associated with genital warts.

While most of these infections will remain subclinical for life, a subset of approximately 13-15 HPV genotypes can lead in rare cases to development of invasive cancer at the site of infection (9, 10). The cervix is most susceptible to HPV-mediated carcinogenesis, with 11,818 cases of cervical cancer occurring in 2010 in the United States (11). While 100% of all cervical cancer cases are attributable to HPV infection, proportions of other cancers attributable to HPV infection vary from 3% of mouth cancer, 12% of oropharyngeal cancer, 40% of cancers of external genitalia (penile and vulvar/vaginal), to 90% of anal cancers (5, 12). Globally, the burden of cervical, penile, anal, vulvar/vaginal, mouth, and oropharyngeal cancers was estimated in 2002 to be over

5.8 million cases among males and over 5.06 million cases among females (12). High-risk types 16 and 18 are estimated to cause nearly 72% of all cancers attributable to HPV and almost 70% of cervical cancer cases (12).

Natural history of HPV

Genital HPV infection is the most common sexually transmitted infection, with an estimated annual incidence of 6.2 million among persons aged 15 to 44 years in the United States (13). Studies have demonstrated that up to 80% of sexually active adults will acquire at least one HPV type infection during their lifetime (14). HPV infection affects 26.8% of women of reproductive age at any given time (15), and, generally, co-infection with multiple HPV types is common (about half of all infections) (16).

While HPV is highly prevalent, most HPV infections are transient in nature; typically, almost half of HPV infections will be cleared or controlled below the limit of detection within six months and 90% of HPV infections will become undetectable within two years of acquisition (17). Among women in the general population, following initial exposure, HPV establishes infection in the basal epithelial layer of the cervix, and is typically cleared or controlled by an intact immune system through cell-mediated immune responses within one to two years of initial infection (18-20); even high-risk type infections typically remain continuously detectable for 12 to 18 months (21). Whether the immunity is sterilizing (e.g., results in viral eradication) or merely controls virus below limits of detection, similar to other human DNA virus infections, remains unclear, though an increasing body of evidence supports the possibility of lifelong latency and immune control (22-25).

Of all HPV infections, approximately ten percent are persistently detectable for more than two years (26) and can cause cytological changes. In a large, multicenter study of U.S. women, while over 90% of prevalent HPV infections cleared within two years, the infections that persisted past 24 months were more likely to continue to persist (20). One explanation for this association may be that prevalent infections have successfully evaded the immune system in order to remain replicative and detectable, while incident infection may reflect new acquisition (27). High-risk HPV types are more likely to persist than low-risk types, and are more likely to be associated with risk of higher-grade cervical lesions (17). These persistent infections need careful clinical management, including follow-up and treatment, to prevent cervical cancer.

Natural history of cervical intraepithelial neoplasia (CIN)

Cervical cytologic abnormalities are classified by histology and by cytology using standard nomenclature to describe the severity of cellular changes induced by HPV infection and HPV-mediated cellular transformation. The nomenclature varies depending on whether cytological examination of exfoliated cells or histological examination of tissue sections is performed. Histology, based on examination of biopsied cervical tissue, is assigned according to range of cellular atypia confined to the epithelium and termed cervical intraepithelial neoplasia (CIN). CIN is classified according to level of dysplasia observed in the tissue sample, from mild (CIN 1), moderate (CIN2), to severe (CIN 3), which are considered high-grade precursors to invasive cervical cancer (28). Squamous intraepithelial lesions (SIL) are detected by cytologic Papanicolau (Pap) smear, a clinical method of cervical cancer screening where cervical cells are sampled, examined under magnification, and classified based on the presence or absence of cellular abnormalities

using standardized terminology according to the Bethesda System (29, 30). Using the Bethesda System, cervical lesions are divided into two grades: low-grade SIL (LSIL) which consists of flat condylomatous changes (indicating HPV presence) and high-grade SIL (HSIL) which are defined as more severe neoplastic lesions (30, 31). The severity of HPV-mediated cell changes from normal cervical epithelium to invasive cervical cancer may be described using either cytology or histology; Figure 1 displays the relationship and overlap between the two classification systems, where LSIL is equivalent to mild CIN, or CIN 1, and HSIL may be CIN 2 or CIN 3, and describes the cellular changes that lead to invasive cervical cancer (32). In brief, HPV initially infects the basal epithelial cells through micro-abrasions or micro-trauma. Viral replication occurs within these basal cells undergoing active cell division; the cytopathic changes that become detectable including koilocytosis, multinucleation, and nuclear enlargement reflect the viral replication occurring in the upper epithelial layer of cells (33). As the upper epithelium is shed, HPV is released and can start a new infection in another host. The cytopathic changes due to productive HPV replication are detected as low-grade squamous intraepithelial lesions, or LSIL (34) and are consistent with CIN1. A small proportion of high-risk HPV infections will persist and cause more severe cytological changes termed high-grade SIL, or HSIL, and is equivalent to CIN grade 2 and 3 (CIN2, CIN3). Finally, the last and uncommon step to invasive cervical cancer is the progression of HSIL or CIN3, which is frequently but not always associated with integration of the HPV genome into host chromosomes (35, 36).

Pap smear tests have been in widespread use for the past few decades in screening programs in the United States to detect early precancerous cervical lesions and thus

prevent invasive cervical cancer (37). Over 60 million Pap tests are performed annually in the United States (16), and prior studies have determined the proportion of various cytological diagnoses that were HPV positive: 49% of ASCUS, 83% of LSIL, and 92% of HSIL (CIN2+) in a U.S. multicenter, community-based population (38); 70% of LSIL, 89% of HSIL in a northern California cohort (39); and high-risk HPV type positive for 66% of CIN1, 83% of CIN2, 93% of CIN3 in a U.S. multicenter screening population (40). Increasing severity of clinical disease tends to reflect increases in proportion of HPV-positive results. Other abnormal cytology results involve glandular cells instead of epithelial cells, and include diagnoses of atypical glandular cells (AGC), AGC favor neoplasia, and endocervical adenocarcinoma in situ (AIS), which are frequently due to benign conditions, but can also be associated with neoplasia and cervical cancer (16).

The majority (~90%) of abnormal Pap diagnoses represent minimally abnormal changes associated with HPV infection (e.g., atypical squamous cells of undetermined significance (ASCUS) and LSIL). Clinical management of abnormal Pap results follows a logic of "equal management of equal risks" based on data on cervical precancer management from a very large cohort of nearly one million women in an integrated health care system followed for over nine years (41). Abnormal Pap results of ASCUS in the absence of HPV testing are followed by repeat cytology one year later (42), as the estimated risk of CIN3+ (as a marker of pre-cancer) is very low (around 2.6%) among these women (41). If ASCUS Pap results are followed with HPV co-testing, and a woman is found to be ASCUS-positive and HPV-negative, the American Society for Colposcopy and Cervical Pathology (ASCCP) recommends extending the repeat Pap and HPV co-test interval to three years for these low-risk women (42).

Low-grade lesions, LSIL or CIN1, represent productive HPV infection, are usually self-limited, and will regress without any treatment (43, 44). However, based on recent research (41), the five-year risk of CIN3+ is 5.2% when LSIL is detected; thus, findings of LSIL-positive/HPV-positive and LSIL-positive/HPV-unknown are both managed with immediate colposcopy. In addition, a positive HPV co-test result with ASCUS Pap finding confers 6.8% risk of CIN3+, which is greater than the 5.2% risk estimated with a LSIL Pap result (41); therefore, the ASCCP also recommends management using immediate colposcopy (42), the typical management strategy when LSIL is detected.

A small subset of SIL will progress to invasive cancer over an average of ten to 15 years (45-47). In the Kaiser Permanente Northern California cohort, there was 30-49% risk of progression from to CIN3+ within five years when HSIL was detected on Pap smear (48). As a result, detection of HSIL on a Pap test will prompt immediate excision or colposcopy (42, 48). These HSIL+ and CIN2+ are the targets of cervical cancer screening, as excisional removal of these lesions is relatively simple and treatment has >90% success rate (49, 50).

Critical role of host immunity on HPV and CIN natural history

It is clear that the nature of the host immune response to HPV infection plays a crucial role in determining which infections develop into long-term persistent infections with higher risk of progression to neoplasia and cancer. The clearance or control of the majority (80-90%) of HPV infections is thought to be facilitated by CD4+ T cell dependent mechanisms, based largely on observations of intraepithelial infiltrates observed during lesion resolution in animal papillomavirus models (51-53). In samples of

varying severity of cervical intraepithelial neoplasia, it has been shown that the density of T cell lymphocytes may play an important role on risk of progression and regression of cervical lesions (54).

In addition to the evidence provided by animal and tissue models, the higher risk of persistent HPV infection, incident HPV infection, and disease progression observed in immune-suppressed populations further support the critical role of host immune response in HPV-associated disease risk. For example, HPV-associated neoplasias have been commonly reported among immunosuppressed transplant recipients (55-58) and incidence of HPV-associated cancers are increased in transplant patients (59). Since transplant recipients are iatrogenically immune-suppressed to prevent rejection of the transplanted organ, it is likely that immune suppression drives HPV persistence and increased cervical cancer risk in this context. Among aging populations, immune senescence is suggested to play a role in HPV persistence (23, 60); the increased risk of HPV-associated outcomes may be explained by waning immunity and inability to maintain control of HPV replication.

Further support for an immunologic role in the natural history of HPV infection comes from the epidemiologic association of HIV infection with higher prevalence and incidence of HPV infection and related cervical disease (61-63). Among HIV-immunosuppressed populations, there is a disproportionate increase in the prevalence of low-grade lesions (62, 64-66), suggesting that immune suppression may increase the risk of clinical evidence of HPV infection and persistence. Epidemiologic studies measuring HPV incidence, prevalence, and persistence have reported a dose-response relationship with increasing immune suppression, measured by both decreasing systemic CD4 count

and increasing systemic HIV viral load (67-70), and increasing severity of HPV-associated clinical disease (71-73). In the Women's Interagency HIV Study (WIHS), a natural history study conducted among HIV-infected and at-risk HIV-uninfected women in the United States, HIV-related immune markers like serostatus, CD4 cell count, and HIV RNA level were found to be associated with abnormal Pap results (74, 75). Infection with multiple HPV types is also more common among HIV-infected populations compared to their uninfected counterparts (76), and, among HIV-positives, multiple type infection is more common with decreasing CD4 cell count (77), offering further support for the critical role of intact cell-mediated immunity in the control of HPV infection. Cervical cancer, representing the fulminant clinical endpoint of uncontrolled, persistent HPV infection, has also been reported to be higher among HIV-infected women, especially those who are severely immunosuppressed (78-80).

Local genital tract immune responses may be even more critical in the control of HPV infections and prevention of disease progression than systemic immune responses. Most of the markers of immune suppression in HIV-infected women are based on systemic measures of immune response. Conversely, HPV infection has no systemic phase (6) and it is likely that the local cervical immune microenvironment plays a critical role in shaping the immune response to HPV infection. Direct measures of HPV-specific cell-mediated immunity are currently unavailable (81); however, more global measures of epithelial immune suppression have been associated with an increased risk of incident HR-HPV detection and prevalent SIL(82). These studies measured T-cell-dependent immune response in the cutaneous epithelium by challenging the participant with common antigens (e.g., *Candida albicans*, tetanus toxoid, and mumps) injected

intradermally on the forearm using the Mantoux method (82, 83). The size of induration, defined as the hard, dense, raised formation just under the skin surface formed by the recruitment of macrophages, monocytes, lymphocytes, and other cellular components by memory T cells in reaction to the antigens, determines the level of delayed type hypersensitivity (DTH) response and is measured between 48 to 72 hours following administration (84-86). Most manufacturers suggest induration of 5mm or greater as the definition of a "positive" test, representing "normal" DTH function. Since intact cell-mediated immunity (CMI) involves interactions between lymphocytes, macrophages, and cytokines, anergy testing serves as an *in vivo* clinical test to measure the functional presence of T-cell immune competence at the epithelial surface (87). Lack of an adequate immune response to the injected antigens results in anergy (84).

In the WIHS, associations between anergy and both prevalent lesions and incident high-risk HPV held even after the investigators adjusted for systemic markers of HIV-associated immune status (e.g., HIV-RNA level and CD4 T-cell strata) (82), supporting the possibility that local cell-mediated immunity is involved in the control of HPV infection. However, the antigens tested were not HPV antigens, and more direct measures of cervical immune responses are needed to confirm the potential association of local immune control of HPV infection.

Indirect evidence for the role of the cervical immune microenvironment in controlling cervical HPV infection also comes from the examination of women during acute HIV infection, which is known to be associated with a profound loss of CD4 memory at mucosal sites. Two studies have shown that women in this early phase of HIV infection have increased risk of multiple new HPV type infection, further supporting the

notion that local immunologic control of latent HPV infection is a primary regulator of HPV natural history (88, 89).

In addition to HIV co-infection, which is highly immune suppressive, other risk factors associated with HPV persistence and cervical cancer have been shown to have immune suppressive functions, including smoking (90), oral contraceptive (OC) use (91-93), and parity (94, 95). Smoking has been identified in many epidemiologic studies as a risk factor for prevalent (96) and incident HPV (96), CIN3+ (97), and cervical cancer (58, 90, 94, 98). Local cell-mediated immune suppression has been suggested to explain the link between tobacco smoking and increased HPV infection and cervical cancer (94, 99). Parity has been hypothesized to be associated with increased risk through various mechanisms including an immunologic pathway; it is thought that pregnancy-induced hormonal changes may alter the immune response to HPV and increase risk of persistence and progression (94, 95). Hormonal modulation of host immunity may also contribute to increased risk of HPV persistence (93), HSIL, and cervical cancer among women who have used OC's for an extended period, usually longer than five years (94), although the biological mechanism has not yet been elucidated. Taken together, these findings lend support for an immune suppressive pathway leading to increased risk of HPV persistence and associated lesions, and indicate the importance of determining whether other risk groups, such as drug users, are at increased risk for HPV and associated disease through a similar immunologic mechanism.

Illicit drug use and HPV/CIN risk

Use of illicit drugs may be potentially useful as a risk stratifier to identify populations likely to progress more rapidly to advanced stage disease and to target

appropriately with screening and treatment to prevent cervical cancer. Prior studies have identified injection drug use, particularly use of cocaine, as a risk factor for HPV infection among both HIV-seropositive and HIV-seronegative women (100, 101). Higher frequency of invasive cervical cancer has been observed among IDUs (102). Table 1 illustrates the overall findings from seven epidemiologic studies published in the past 18 years that evaluated the association between illicit drug use and HPV and associated disease outcomes. Overall, the existing literature is consistent with an increased risk of HPV infection and related clinical outcomes among populations who use illicit drugs, with estimates of association ranging in magnitude from odds ratios of 1.3 to 2.2 for HPV infection (100, 101, 103) to over two-fold increases for Pap abnormalities among HIV immunosuppressed (CD4<200) recent crack cocaine users (100) and drug users (104). Cervical cancer risk among drug users has also been observed to be elevated (79, 102, 104, 105). However, the majority of these epidemiologic studies have defined drug exposures broadly as being an injection drug user or having drug addiction, (79, 102-105), making it difficult to infer effects of exposure to specific drugs. Prior research has also been hampered by insufficient control of potential confounders, including not accounting for sexual risk behavior (103, 105), presenting only unadjusted analyses (104), as well as defining the drug exposure using non-specific measures like diagnostic codes from inpatient admissions (105). Few studies were prospectively designed and included relevant measures of potential confounders to appropriately examine the relationship between cocaine use and HPV infection and related outcomes.

Published literature to date has supported two potential mechanisms to explain the association between drug use and HPV infection: direct effects of illicit drug exposures

and the indirect effects of sexual behaviors that are associated with both illicit drug use and HPV infection (34, 106). However, the limited body of literature has not yet identified the pathway to explain the link.

Since injection drug use is associated with increases in risky sexual behavior (107, 108), the observed association with HPV infection may merely result from confounding by high-risk sexual behavior. However, three studies have also suggested that sexual behavior including, but not limited to, recent (i.e., in last six months) and lifetime number of male partners (100, 101) and prostitution (102) does not completely explain the association between drug use and HPV-associated outcomes. Since the associations between HPV infection and drug use were not substantially attenuated after adjustment for several markers of sexual activity and sexual risk (100), we cannot rule out the possibility that the observed effect may in fact have a biological basis.

Evidence supporting a biological mechanism of drug-induced immune suppression originates at the cellular level. Cocaine is a sigma-ligand agonist and is known to induce immune suppression through suppressing proliferation of lymphocytes, including alteration of natural killer cell, helper T cell (CD4+), and cytotoxic T cell (CD8+) activity, as well as neutrophil and macrophage activity as the first line of defense against infection (109). There is convincing laboratory evidence for immune suppressive effects caused by cocaine exposure including a potential cocaine-facilitated switch to a Th2 anti-inflammatory immune response associated with increased susceptibility to infection with bacteria, protozoa, and viruses (110). Anti-HPV effector T cells prevent growth of HPV-associated tumors in various animal models (111) and a Th2 immune response with low levels of IFN-gamma and increased IL-10 production fails to protect

and control HPV infection and its oncogenic effects, including *in vivo* tumor growth (110). Additional immune suppressive effects associated with cocaine exposure include reduced delayed-type hypersensitivity (DTH) response and reduced effector cell proliferation (112).

In addition to *in vitro* studies, animal models demonstrate additional support for cocaine-induced immune suppression specific to mucosal and epithelial sites, which may have a direct impact on epithelia-tropic HPV. Murine models have shown cocaine exposure to be associated with a decrease in IgA+ cells and an increase in number of CD8+ cells in the intestinal lamina propria, suggesting injury to mucosal immunity (113). Oral cocaine administration appears to inhibit cell-mediated immunity at epithelial surfaces, as measured by the DTH response to dinitrofluorobenzene (DNFB) in the mouse model (114). Repeated daily injection of cocaine in the mouse model induced a diminished DTH reaction and a slight decrease in IgG levels, suggesting reduced mucosal immunity associated with chronic cocaine exposure (115).

Despite growing evidence for the immunosuppressive effects of illicit drugs like cocaine in animal and *in vitro* studies, there is a lack of longitudinal epidemiologic studies to demonstrate the effect of cocaine and other illicit drugs on increased incidence of infections in humans (110, 112, 116). The only large epidemiologic studies that have examined drug use and HPV are two prospective cohort studies among women with HIV infection or at high-risk for HIV infection as a result of injection drug use or who were current or former injection drug users, the WIHS and ALIVE studies, have evaluated the association between drug use and HPV and associated disease outcomes (100, 101). In these studies, recent (defined as occurring within the previous six months) crack/cocaine

use was associated with a 1.7 fold-increased risk of new HPV infection in the ALIVE cohort (100) and 1.3 fold-increased risk of oncogenic HPV type infection in the WIHS cohort (101). In the WIHS, increased risk of oncogenic HPV-positive cervical lesions was also observed among crack/cocaine users (OR 1.70, 95% C.I.: 1.27-2.27) (101). In the ALIVE study, an increased risk of Pap abnormality was observed only among the most severely immune suppressed HIV-infected women (OR 2.4, 95% C.I: 1.1-5.1) (100)who recently used crack cocaine. However, this prior analysis was limited to a subset of the overall cohort enrolled in a gynecological sub-study and to only five years of follow-up time. Evidence supporting cocaine-induced immune suppression has been reported among HIV-infected drug users, where both persistent and intermittent crack cocaine (a crystallized form of cocaine that is taken by inhalation) users were more likely than non-users to develop new AIDS-defining illnesses, as well as lower CD4 counts and higher HIV-1 RNA levels (117), suggesting that concurrent crack use may accelerate HIV-mediated immune suppression. Understanding the relationship between cocaine exposure and immune suppression still requires further examination in larger cohorts of drug using women with longer follow-up.

Conceptual Framework

In summary, HPV infection has been found to be associated with both cutaneous anergy and cocaine/crack use, independent of sexual behavior and HIV-related immune markers (HIV RNA levels and CD4 counts). Since animal and tissue models have shown that use of illicit drugs modulates the immune response, which may result in anergy, we hypothesize that the association between cocaine/crack use and HPV is mediated by drug-induced reduction in DTH response.

The analyses conducted in this dissertation will allow direct assessment of the association between cocaine use and cutaneous anergy as a marker of impaired epithelial cell-mediated immune response, and will explore whether cocaine use is associated with increases in HPV-associated cervical cytological abnormalities. Figure 2 shows the fundamental conceptual framework in which we examine associations between use of cocaine and HPV. We test the hypothesis that the use of crack/cocaine is associated with dampened local epithelial immunity, measured by the absence of a DTH response using the cutaneous anergy skin test (Chapter Two). Additionally, there is some evidence that, among injection drug using populations, exposure to cocaine/crack cocaine may be associated with increased HPV infection (100). To better understand this purported relationship, we extend the previous analyses in a current and former drug-using community-based cohort with over 18 years of follow-up to examine the association between cocaine use and clinically detectable HPV infection (Chapter Three).

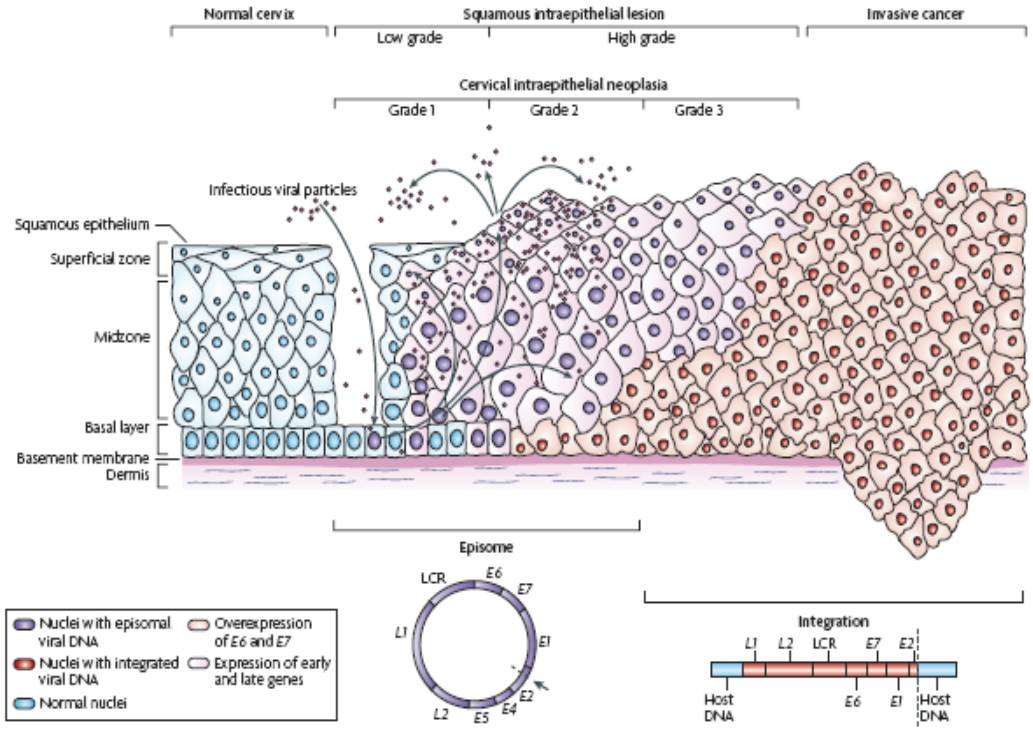
Analysis/Specific Aims

In order to advance our understanding of the relationships between HPV, immune status, and cocaine use among women with HIV or at high-risk of acquiring HIV, we reviewed relevant literature and conducted the following analyses:

1. We examined the association between recent use of cocaine and incident HPV-associated cytological abnormalities in an urban-dwelling cohort of former and current injection drug using women with and without HIV infection.
2. We evaluated whether an immunosuppressive pathway might explain the high rate of HPV-associated outcomes among drug users by assessing the relationship

between cocaine use and a measure of delayed-type hypersensitivity immune responsiveness, cutaneous anergy, in a longitudinal, urban cohort of HIV-infected and –uninfected women across the United States.

Figure 1. HPV-mediated progression to cervical cancer



Source: Woodman et al. *Nature Reviews Cancer* 7, 11-22 (January 2007)

Figure 2. Conceptual Framework for the Associations between Cocaine Use, Immune suppression, and HPV infection

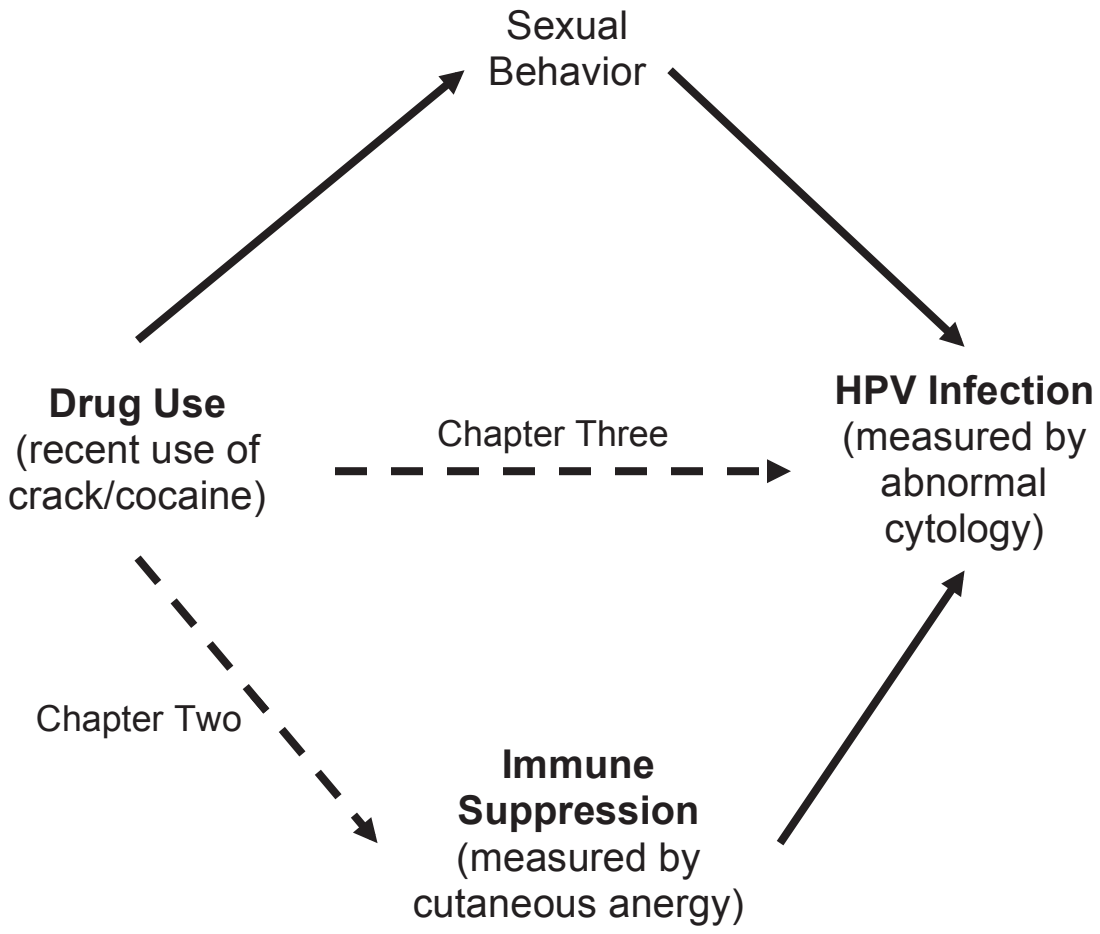


Table 1. Epidemiologic studies evaluating risk of HPV-associated clinical outcomes associated with illicit drug use

Author Year	Study Design	Study Population	Drug Exposure Categorization	Outcome Definition	Confounders	Major Findings
Serraino 1996	Registry-based retrospective cohort	Women with AIDS in Italy, 1993-1995	Mode of HIV acquisition via injection drug use (IDU)	Invasive cervical cancer	Age, marital status	OR 2.6 (95% CI 1.2-5.3) compared to women with AIDS who acquired HIV through heterosexual intercourse
Serraino 1999	Longitudinal cohort	IDU women from three cohorts of female IDU between 15-49 years old in France and Italy	IDU	Invasive cervical cancer	Stratified by HIV serostatus, age	Among HIV-positive IDU, SIR = 16.7 compared to heterosexual women (SIR = 6.7). No cases of ICC were diagnosed among HIV-negative IDU women (0.15 cases expected).
Reece 2007	Cross-sectional	155 substance use disorder and 77 general medical patients between 15-45 years old seen in primary care setting	Addiction defined by opiate dependence (current use of heroin or morphine and in treatment with methadone) or other illicit substance (amphetamine or cannabis)	(i) Abnormality; (ii) CIN2/3; (iii) CIN2-cervical cancer	None (only provided results from unadjusted analyses)	(i) OR 2.19 (95% CI 1.03-4.73); (ii) OR 3.71 (95% CI 1.04-20.04); (iii) OR 4.82 (95% CI 1.39-25.64)

Author Year	Study Design	Study Population	Drug Exposure Categorization	Outcome Definition	Confounders	Major Findings
Minkoff 2008	Prospective cohort	Women's Interagency HIV Study (2,584 HIV+ and 915 HIV- women)	Self-reported recent use of crack/cocaine in the last 6 months	Prevalence, incidence, and clearance of HPV, oncogenic HPV, nononcogenic HPV, and oncogenic SIL	Adjusted for age, race, HIV serostatus, CD4+ T-cell count, sexual partners during prior 6 months, and smoking behavior	OR 1.30 (95% CI 1.09-1.55) for oncogenic HPV; OR 1.70 (95% CI 1.27-2.27) for SIL with oncogenic HPV; HR 1.51 (95% CI 0.99-2.30) for incident SIL with oncogenic HPV; HR 0.57 (95% CI 0.34-0.97) for clearance of oncogenic+ SIL
Syrjänen 2008	(a) Nested case-control; (b) prospective cohort	Latin American Screening Study (Subset of 109 cases and 436 controls out of 12,114 women in Brazil and Argentina)	Self-reported drug addiction (Regular use of cannabis, cocaine/crack, solvents, heroin, LSD, and morphine)	(i) High-risk HPV infection; (ii) Prevalent CIN2+	Matched on age	(a) (i) OR 2.15 (95% CI 1.17-3.94) (b) (i) OR 0.70 (95% CI 0.31-1.58) (b) (ii) OR 3.42 (95% CI 0.65-17.89)

Author Year	Study Design	Study Population	Drug Exposure Categorization	Outcome Definition	Confounders	Major Findings
Phelan 2009	Prospective cohort	AIDS Link to Intravenous Experience (ALIVE) study (146 HIV+ and 73 HIV- injection drug using women in Baltimore, MD)	Self-reported crack (cocaine), marijuana, heroin use in last 6 months	(i) Newly detected HPV infection; (ii) Pap abnormality	Adjusted for HIV serostatus, age, number of male sex partners (in the last 10 years for overall cohort, in the last 6 months among HIV-uninfected)	(i) Among overall cohort: OR 1.7, 95% CI 1.1-2.6 for crack; Among HIV-uninfected: OR 3.5, 95% CI 1.3-9.5 for marijuana; (ii) Among HIV-infected & CD4<200 cells/mm^3: OR 2.4, 95% CI 1.1-5.1 for crack
Kricker 2013	Nested case-control	(i) 6,523 cases and 65,230 controls; (ii) 239 cases and 2,390 controls; hospitalized women between 20-54 years old in New South Wales, 2000-2006	Illicit drug use (based on diagnosis codes from hospital admission for presence of problematic drug use)	(i) CIN2/3; (ii) cervical cancer	Matched on age and year of hospital admission; adjusted for years of Pap tests, current and former smoking	(i) OR 1.13 (95% CI 1.04-1.23); (ii) OR 1.43 (95% CI 0.96-2.15)

References

1. Burk RD, Harari A, Chen Z. Human papillomavirus genome variants. Virology. 2013;445(1-2):232-43.
2. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70-9.
3. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004;324(1):17-27.
4. Gottschling M, Goker M, Stamatakis A, Bininda-Emonds OR, Nindl I, Bravo IG. Quantifying the phylodynamic forces driving papillomavirus evolution. Molecular biology and evolution. 2011;28(7):2101-13.
5. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol. 2010;117(2 Suppl):S5-10.
6. Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clinical microbiology reviews. 2012;25(2):215-22.
7. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55-70.
8. Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T, et al. Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis. 2009;9(6):347-56.
9. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12-9.
10. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348(6):518-27.
11. U.S. Cancer Statistics Working Group. United States Cancer Statistics: 1999–2010 Incidence and Mortality Web-based Report. Atlanta (GA): Department of Health and Human Services, Centers for Disease Control and Prevention, and National Cancer Institute; 2013. Available at: http://www.cdc.gov/uscs.
12. Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. Vaccine. 2006;24 Suppl 3:S3/11-25.
13. Weinstock H, Berman S, Cates W, Jr. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect Sex Reprod Health. 2004;36(1):6-10.
14. Brown DR, Shew ML, Qadadri B, Neptune N, Vargas M, Tu W, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis. 2005;191(2):182-92.
15. Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, et al. Prevalence of HPV infection among females in the United States. Jama. 2007;297(8):813-9.
16. Wheeler CM. Natural history of human papillomavirus infections, cytologic and histologic abnormalities, and cancer. Obstet Gynecol Clin North Am. 2008;35(4):519-36; vii.

17. Moscicki AB, Schiffman M, Burchell A, Albero G, Giuliano AR, Goodman MT, et al. Updating the natural history of human papillomavirus and anogenital cancers. Vaccine. 2012;30 Suppl 5:F24-33.
18. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006;24 Suppl 1:S1-15.
19. Richardson H, Kelsall G, Tellier P, Voyer H, Abrahamowicz M, Ferenczy A, et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2003;12(6):485-90.
20. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM, Group A. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J Infect Dis. 2007;195(11):1582-9.
21. Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Desy M, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis. 1999;180(5):1415-23.
22. Gravitt PE. Evidence and impact of human papillomavirus latency. The open virology journal. 2012;6:198-203.
23. Gonzalez P, Hildesheim A, Rodriguez AC, Schiffman M, Porras C, Wacholder S, et al. Behavioral/lifestyle and immunologic factors associated with HPV infection among women older than 45 years. Cancer Epidemiol Biomarkers Prev. 2010;19(12):3044-54.
24. Doorbar J. Latent papillomavirus infections and their regulation. Current opinion in virology. 2013;3(4):416-21.
25. Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst. 2005;97(8):577-86.
26. Stanley M. Immunobiology of HPV and HPV vaccines. Gynecol Oncol. 2008;109(2 Suppl):S15-21.
27. Rodriguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. J Natl Cancer Inst. 2010;102(5):315-24.
28. Wright TC, Jr., Denny L, Kuhn L, Goldie S. Use of visual screening methods for cervical cancer screening. Obstet Gynecol Clin North Am. 2002;29(4):701-34.
29. Tiro JA, Saraiya M, Jain N, Liddon N, Cokkinides V, Lai SM, et al. Human papillomavirus and cervical cancer behavioral surveillance in the US. Cancer. 2008;113(10 Suppl):3013-30.
30. Solomon D. The Bethesda System for reporting cervical/vaginal cytologic diagnosis: an overview. Int J Gynecol Pathol. 1991;10(4):323-5.
31. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. Jama. 2002;287(16):2114-9.
32. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007;7(1):11-22.

33. Erickson BK, Alvarez RD, Huh WK. Human papillomavirus: what every provider should know. Am J Obstet Gynecol. 2013;208(3):169-75.
34. Steben M, Duarte-Franco E. Human papillomavirus infection: epidemiology and pathophysiology. Gynecol Oncol. 2007;107(2 Suppl 1):S2-5.
35. Vinokurova S, Wentzensen N, Kraus I, Klaes R, Driesch C, Melsheimer P, et al. Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. Cancer Res. 2008;68(1):307-13.
36. Melsheimer P, Vinokurova S, Wentzensen N, Bastert G, von Knebel Doeberitz M. DNA aneuploidy and integration of human papillomavirus type 16 e6/e7 oncogenes in intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix uteri. Clinical cancer research : an official journal of the American Association for Cancer Research. 2004;10(9):3059-63.
37. Datta SD, Koutsky LA, Ratelle S, Unger ER, Shlay J, McClain T, et al. Human papillomavirus infection and cervical cytology in women screened for cervical cancer in the United States, 2003-2005. Ann Intern Med. 2008;148(7):493-500.
38. Group A-LTS. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol. 2003;188(6):1383-92.
39. Manos MM, Kinney WK, Hurley LB, Sherman ME, Shieh-Ngai J, Kurman RJ, et al. Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results. JAMA. 1999;281(17):1605-10.
40. Wright TC, Jr., Stoler MH, Behrens CM, Apple R, Derion T, Wright TL. The ATHENA human papillomavirus study: design, methods, and baseline results. Am J Obstet Gynecol. 2012;206(1):46 e1- e11.
41. Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, et al. Benchmarking CIN 3+ risk as the basis for incorporating HPV and Pap cotesting into cervical screening and management guidelines. J Low Genit Tract Dis. 2013;17(5 Suppl 1):S28-35.
42. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17(5 Suppl 1):S1-S27.
43. Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol. 1998;92(4 Pt 2):727-35.
44. Winer RL, Kiviat NB, Hughes JP, Adam DE, Lee SK, Kuypers JM, et al. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis. 2005;191(5):731-8.
45. Vink MA, Bogaards JA, van Kemenade FJ, de Melker HE, Meijer CJ, Berkhof J. Clinical progression of high-grade cervical intraepithelial neoplasia: estimating the time to preclinical cervical cancer from doubly censored national registry data. Am J Epidemiol. 2013;178(7):1161-9.
46. van Oortmarssen GJ, Habbema JD. Duration of preclinical cervical cancer and reduction in incidence of invasive cancer following negative pap smears. Int J Epidemiol. 1995;24(2):300-7.

47. Holowaty P, Miller AB, Rohan T, To T. Natural history of dysplasia of the uterine cervix. J Natl Cancer Inst. 1999;91(3):252-8.
48. Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, et al. Five-year risks of CIN 3+ and cervical cancer among women with HPV-positive and HPV-negative high-grade Pap results. J Low Genit Tract Dis. 2013;17(5 Suppl 1):S50-5.
49. Nuovo J, Melnikow J, Willan AR, Chan BK. Treatment outcomes for squamous intraepithelial lesions. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2000;68(1):25-33.
50. Wright TC, Jr., Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D, et al. 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. Am J Obstet Gynecol. 2007;197(4):340-5.
51. Nicholls PK, Klaunberg BA, Moore RA, Santos EB, Parry NR, Gough GW, et al. Naturally occurring, nonregressing canine oral papillomavirus infection: host immunity, virus characterization, and experimental infection. Virology. 1999;265(2):365-74.
52. Nicholls PK, Moore PF, Anderson DM, Moore RA, Parry NR, Gough GW, et al. Regression of canine oral papillomas is associated with infiltration of CD4+ and CD8+ lymphocytes. Virology. 2001;283(1):31-9.
53. Wilgenburg BJ, Budgeon LR, Lang CM, Griffith JW, Christensen ND. Characterization of immune responses during regression of rabbit oral papillomavirus infections. Comparative medicine. 2005;55(5):431-9.
54. Monnier-Benoit S, Mauny F, Riethmuller D, Guerrini JS, Capilna M, Felix S, et al. Immunohistochemical analysis of CD4+ and CD8+ T-cell subsets in high risk human papillomavirus-associated pre-malignant and malignant lesions of the uterine cervix. Gynecol Oncol. 2006;102(1):22-31.
55. Hinten F, Meeuwis KA, van Rossum MM, de Hullu JA. HPV-related (pre)malignancies of the female anogenital tract in renal transplant recipients. Critical reviews in oncology/hematology. 2012;84(2):161-80.
56. Halpert R, Fruchter RG, Sedlis A, Butt K, Boyce JG, Sillman FH. Human papillomavirus and lower genital neoplasia in renal transplant patients. Obstet Gynecol. 1986;68(2):251-8.
57. Schneider V, Kay S, Lee HM. Immunosuppression as a high-risk factor in the development of condyloma acuminatum and squamous neoplasia of the cervix. Acta Cytol. 1983;27(3):220-4.
58. Dugue PA, Rebolj M, Garred P, Lynge E. Immunosuppression and risk of cervical cancer. Expert review of anticancer therapy. 2013;13(1):29-42.
59. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007;370(9581):59-67.
60. Garcia-Pineres AJ, Hildesheim A, Herrero R, Trivett M, Williams M, Atmetlla I, et al. Persistent human papillomavirus infection is associated with a generalized decrease in immune responsiveness in older women. Cancer Res. 2006;66(22):11070-6.
61. Ho GY, Burk RD, Fleming I, Klein RS. Risk of genital human papillomavirus infection in women with human immunodeficiency virus-induced immunosuppression. Int J Cancer. 1994;56(6):788-92.
62. Duerr A, Kieke B, Warren D, Shah K, Burk R, Peipert JF, et al. Human papillomavirus-associated cervical cytologic abnormalities among women with or at risk

of infection with human immunodeficiency virus. Am J Obstet Gynecol. 2001;184(4):584-90.
63. Sun XW, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC, Jr. Human papillomavirus infection in women infected with the human immunodeficiency virus. N Engl J Med. 1997;337(19):1343-9.
64. Massad LS, Ahdieh L, Benning L, Minkoff H, Greenblatt RM, Watts H, et al. Evolution of cervical abnormalities among women with HIV-1: evidence from surveillance cytology in the women's interagency HIV study. J Acquir Immune Defic Syndr. 2001;27(5):432-42.
65. Massad LS, Riester KA, Anastos KM, Fruchter RG, Palefsky JM, Burk RD, et al. Prevalence and predictors of squamous cell abnormalities in Papanicolaou smears from women infected with HIV-1. Women's Interagency HIV Study Group. J Acquir Immune Defic Syndr. 1999;21(1):33-41.
66. Wright TC, Jr., Koulos J, Schnoll F, Swanbeck J, Ellerbrock TV, Chiasson MA, et al. Cervical intraepithelial neoplasia in women infected with the human immunodeficiency virus: outcome after loop electrosurgical excision. Gynecol Oncol. 1994;55(2):253-8.
67. Heard I, Tassie JM, Schmitz V, Mandelbrot L, Kazatchkine MD, Orth G. Increased risk of cervical disease among human immunodeficiency virus-infected women with severe immunosuppression and high human papillomavirus load(1). Obstet Gynecol. 2000;96(3):403-9.
68. Ahdieh L, Klein RS, Burk R, Cu-Uvin S, Schuman P, Duerr A, et al. Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. J Infect Dis. 2001;184(6):682-90.
69. Zeier MD, Botha MH, van der Merwe FH, Eshun-Wilson I, van Schalkwyk M, la Grange M, et al. Progression and persistence of low-grade cervical squamous intraepithelial lesions in women living with human immunodeficiency virus. J Low Genit Tract Dis. 2012;16(3):243-50.
70. Nappi L, Carriero C, Bettocchi S, Herrero J, Vimercati A, Putignano G. Cervical squamous intraepithelial lesions of low-grade in HIV-infected women: recurrence, persistence, and progression, in treated and untreated women. European journal of obstetrics, gynecology, and reproductive biology. 2005;121(2):226-32.
71. Boardman LA, Cotter K, Raker C, Cu-Uvin S. Cervical intraepithelial neoplasia grade 2 or worse in human immunodeficiency virus-infected women with mildly abnormal cervical cytology. Obstet Gynecol. 2008;112(2 Pt 1):238-43.
72. Cohn JA, Gagnon S, Spence MR, Harrison DD, Kluzak TR, Langenberg P, et al. The role of human papillomavirus deoxyribonucleic acid assay and repeated cervical cytologic examination in the detection of cervical intraepithelial neoplasia among human immunodeficiency virus-infected women. Cervical Disease Study Group of the American Foundation for AIDS Research Community Based Clinical Trials Network. Am J Obstet Gynecol. 2001;184(3):322-30.
73. Johnson JC, Burnett AF, Willet GD, Young MA, Doniger J. High frequency of latent and clinical human papillomavirus cervical infections in immunocompromised human immunodeficiency virus-infected women. Obstet Gynecol. 1992;79(3):321-7.

74. Minkoff H, Ahdieh L, Massad LS, Anastos K, Watts DH, Melnick S, et al. The effect of highly active antiretroviral therapy on cervical cytologic changes associated with oncogenic HPV among HIV-infected women. Aids. 2001;15(16):2157-64.
75. Massad LS, Xie X, Minkoff H, Darragh TM, D'Souza G, Sanchez-Keeland L, et al. Abnormal Pap Tests and Human Papillomavirus Infections Among HIV-Infected and Uninfected Women Who Have Sex With Women. J Low Genit Tract Dis. 2013.
76. Palefsky JM, Minkoff H, Kalish LA, Levine A, Sacks HS, Garcia P, et al. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. J Natl Cancer Inst. 1999;91(3):226-36.
77. Strickler HD, Palefsky JM, Shah KV, Anastos K, Klein RS, Minkoff H, et al. Human papillomavirus type 16 and immune status in human immunodeficiency virus-seropositive women. J Natl Cancer Inst. 2003;95(14):1062-71.
78. Abraham AG, D'Souza G, Jing Y, Gange SJ, Sterling TR, Silverberg MJ, et al. Invasive cervical cancer risk among HIV-infected women: a North American multicohort collaboration prospective study. J Acquir Immune Defic Syndr. 2013;62(4):405-13.
79. Serraino D, Carrieri P, Pradier C, Bidoli E, Dorrucci M, Ghetti E, et al. Risk of invasive cervical cancer among women with, or at risk for, HIV infection. Int J Cancer. 1999;82(3):334-7.
80. Frisch M, Biggar RJ, Goedert JJ. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. J Natl Cancer Inst. 2000;92(18):1500-10.
81. Wheeler CM. The natural history of cervical human papillomavirus infections and cervical cancer: gaps in knowledge and future horizons. Obstet Gynecol Clin North Am. 2013;40(2):165-76.
82. Harris TG, Burk RD, Xue X, Anastos K, Minkoff H, Massad LS, et al. Association of cutaneous anergy with human papillomavirus and cervical neoplasia in HIV-seropositive and seronegative women. Aids. 2007;21(14):1933-41.
83. Howard TP, Solomon DA. Reading the tuberculin skin test. Who, when, and how? Arch Intern Med. 1988;148(11):2457-9.
84. Anergy skin testing and tuberculosis [corrected] preventive therapy for HIV-infected persons: revised recommendations. Centers for Disease Control and Prevention. MMWR Recomm Rep. 1997;46(RR-15):1-10.
85. Wright PW, Crutcher JE, Holiday DB. Selection of skin test antigens to evaluate PPD anergy. J Fam Pract. 1995;41(1):59-64.
86. CDC. Mantoux Tuberculin Skin Test Videotape Facilitator Guide [cited 2009 3/30/09]. Available from: http://www.cdc.gov/tb/pubs/Mantoux/guide.htm.
87. Rosenstreich DL. Evaluation of delayed hypersensitivity: from PPD to poison ivy. Allergy Proc. 1993;14(6):395-400.
88. Nowak RG, Gravitt PE, Morrison CS, Gange SJ, Kwok C, Oliver AE, et al. Increases in human papillomavirus detection during early HIV infection among women in Zimbabwe. J Infect Dis. 2011;203(8):1182-91.
89. Wang C, Wright TC, Denny L, Kuhn L. Rapid rise in detection of human papillomavirus (HPV) infection soon after incident HIV infection among South African women. J Infect Dis. 2011;203(4):479-86.

90. Plummer M, Herrero R, Franceschi S, Meijer CJ, Snijders P, Bosch FX, et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case--control study. Cancer causes & control : CCC. 2003;14(9):805-14.
91. Moreno V, Bosch FX, Munoz N, Meijer CJ, Shah KV, Walboomers JM, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet. 2002;359(9312):1085-92.
92. Vaccarella S, Herrero R, Dai M, Snijders PJ, Meijer CJ, Thomas JO, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol Biomarkers Prev. 2006;15(11):2148-53.
93. Marks M, Gravitt PE, Gupta SB, Liaw KL, Tadesse A, Kim E, et al. Combined oral contraceptive use increases HPV persistence but not new HPV detection in a cohort of women from Thailand. J Infect Dis. 2011;204(10):1505-13.
94. Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. Journal of the National Cancer Institute Monographs. 2003(31):20-8.
95. Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet. 2002;359(9312):1093-101.
96. Minkoff H, Feldman JG, Strickler HD, Watts DH, Bacon MC, Levine A, et al. Relationship between smoking and human papillomavirus infections in HIV-infected and -uninfected women. J Infect Dis. 2004;189(10):1821-8.
97. McIntyre-Seltman K, Castle PE, Guido R, Schiffman M, Wheeler CM, Group A. Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. Cancer Epidemiol Biomarkers Prev. 2005;14(5):1165-70.
98. Koshiol J, Schroeder J, Jamieson DJ, Marshall SW, Duerr A, Heilig CM, et al. Smoking and time to clearance of human papillomavirus infection in HIV-seropositive and HIV-seronegative women. Am J Epidemiol. 2006;164(2):176-83.
99. Poppe WA, Ide PS, Drijkoningen MP, Lauweryns JM, Van Assche FA. Tobacco smoking impairs the local immunosurveillance in the uterine cervix. An immunohistochemical study. Gynecologic and obstetric investigation. 1995;39(1):34-8.
100. Phelan DF, Gange SJ, Ahdieh-Grant L, Mehta SH, Kirk GD, Shah K, et al. Determinants of Newly Detected Human Papillomavirus Infection in HIV-Infected and HIV-Uninfected Injection Drug Using Women. Sex Transm Dis. 2009.
101. Minkoff H, Zhong Y, Strickler HD, Watts DH, Palefsky JM, Levine AM, et al. The relationship between cocaine use and human papillomavirus infections in HIV-seropositive and HIV-seronegative women. Infect Dis Obstet Gynecol. 2008;2008:587082.
102. Serraino D, Napoli PA, Zaccarelli M, Alliegro MB, Pezzotti P, Rezza G. High frequency of invasive cervical cancer among female injecting drug users with AIDS in Italy. Aids. 1996;10(9):1041-2.
103. Syrjanen K, Naud P, Derchain S, Roteli-Martins C, Longatto-Filho A, Tatti S, et al. Drug addiction is not an independent risk factor for oncogenic human papillomavirus

infections or high-grade cervical intraepithelial neoplasia: case-control study nested within the Latin American Screening study cohort. Int J STD AIDS. 2008;19(4):251-8.
104. Reece A. Lifetime prevalence of cervical neoplasia in addicted and medical patients. The Australian & New Zealand journal of obstetrics & gynaecology. 2007;47(5):419-23.
105. Kricker A, Burns L, Goumas C, Armstrong BK. Cervical screening, high-grade squamous lesions, and cervical cancer in illicit drug users. Cancer causes & control : CCC. 2013;24(7):1449-57.
106. Temmerman M, Tyndall MW, Kidula N, Claeys P, Muchiri L, Quint W. Risk factors for human papillomavirus and cervical precancerous lesions, and the role of concurrent HIV-1 infection. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 1999;65(2):171-81.
107. Celentano DD, Latimore AD, Mehta SH. Variations in sexual risks in drug users: emerging themes in a behavioral context. Curr HIV/AIDS Rep. 2008;5(4):212-8.
108. Vlahov D, Safaien M, Lai S, Strathdee SA, Johnson L, Sterling T, et al. Sexual and drug risk-related behaviours after initiating highly active antiretroviral therapy among injection drug users. Aids. 2001;15(17):2311-6.
109. Baldwin GC, Roth MD, Tashkin DP. Acute and chronic effects of cocaine on the immune system and the possible link to AIDS. J Neuroimmunol. 1998;83(1-2):133-8.
110. Cabral GA. Drugs of abuse, immune modulation, and AIDS. J Neuroimmune Pharmacol. 2006;1(3):280-95.
111. Agarossi A, Casolati E, Valieri M, Ferrazzi E, Maffeis G, Trabattoni D, et al. Mucosal immune response to Human Papilloma Virus (HPV) infection in HIV positive women. Med Wieku Rozwoj. 2003;7(4 Pt 1):495-502.
112. Friedman H, Newton C, Klein TW. Microbial infections, immunomodulation, and drugs of abuse. Clinical microbiology reviews. 2003;16(2):209-19.
113. Lopez MC, Watson RR. Effect of cocaine and murine AIDS on lamina propria T and B cells in normal mice. Life Sci. 1994;54(9):PL147-51.
114. Watson ES, Murphy JC, ElSohly HN, ElSohly MA, Turner CE. Effects of the administration of coca alkaloids on the primary immune responses of mice: interaction with delta 9-tetrahydrocannabinol and ethanol. Toxicol Appl Pharmacol. 1983;71(1):1-13.
115. Starec M, Rouveix B, Sinet M, Chau F, Desforges B, Pocidalo JJ, et al. Immune status and survival of opiate- and cocaine-treated mice infected with Friend virus. J Pharmacol Exp Ther. 1991;259(2):745-50.
116. Friedman H, Pross S, Klein TW. Addictive drugs and their relationship with infectious diseases. FEMS Immunol Med Microbiol. 2006;47(3):330-42.
117. Cook JA, Burke-Miller JK, Cohen MH, Cook RL, Vlahov D, Wilson TE, et al. Crack cocaine, disease progression, and mortality in a multicenter cohort of HIV-1 positive women. AIDS. 2008;22(11):1355-63.

Chapter Two

Cocaine use and immune suppression in HIV-infected and at-risk uninfected women during the pre-HAART era: a possible link to HPV risk

Abstract

Background: Cocaine use has been linked to an increased risk of prevalent and incident HPV infection. Cocaine has also shown to exert immune suppressive effects in laboratory and animal settings. However, cocaine has not yet been examined in populations at high risk for human papillomavirus (HPV) infection and reactivation. The potential influence of cocaine on the role of host immune control and subsequent viral clearance and suppression of viral replication is critical to understanding the natural history of HPV infection.

Objective: To evaluate the association between cocaine use and epithelial immune suppression, measured by cutaneous anergy, in a population of HIV-infected and at-risk HIV-uninfected female participants in a HIV natural history study.

Methods: We conducted a cross-sectional analysis of 1,373 women enrolled in a prospective cohort study at six U.S. sites with an anergy test completed in the pre-HAART era (before July 1996). We used logistic regression to model the association between recent use of cocaine and cutaneous anergy, adjusting for age, sexual risk behavior, use of other illicit drugs, employment status, and body mass index, and stratified our results by HIV serostatus.

Results: Overall, 46% (n=635) of women were found to have a diminished immune response indicating anergy. Among HIV-uninfected women, those who reported recent cocaine use had more than double the risk of having an anergic test result compared to non-cocaine-users (OR 2.29, 95% C.I.: 1.06-4.95). However, no association was observed among HIV-infected women.

Conclusion: Recent use of cocaine was found to be associated with decreased epithelial immunity among women without HIV infection, which has important implications for understanding HPV natural history. Moreover, the risk of having an anergic test result was magnified after accounting for sexual risk behaviors, lending support to the hypothesis that immune suppression resulting from cocaine use may in part explain the association between cocaine and new HPV detection.

Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted infection, with an estimated annual incidence of 14 million among persons aged 15 to 44 years in the United States (1). At any given time, HPV infection affects over one-quarter of women of reproductive age (2) and the cumulative prevalence of HPV among adolescent women can be as high as 81% (3). While a minority of high-risk HPV infections can progress to invasive cervical cancer, the vast majority of infections appear to result in a robust immune response leading to loss of HPV DNA detection with 1-2 years of infection (4, 5). While previous convention was that loss of HPV DNA detection indicated complete virologic clearance, increasing evidence suggests that it actually may reflect robust immunologic control of very low copy HPV DNA persistence (or latency) in the basal epithelial cells (6, 7). In light of this new model of HPV natural history, risk factors associated with new HPV detection should be evaluated as potentially increasing both risk of HPV acquisition and/or loss of immunological control of latent HPV.

We have previously reported an increased occurrence of prevalent and incident HPV infection among women using cocaine and/or crack cocaine, independent of sexual risk behaviors, in a long-standing cohort of HIV-infected and uninfected women (Women's Interagency HIV Study [WIHS]) as well as a cohort of HIV-infected and uninfected women with a history of injecting drug use (AIDS Linked to the IntraVenous Experience [ALIVE]) (8, 9). The specificity of the association with cocaine/crack use and the absence of significant attenuation of risk after sexual behavior adjustment suggest that this association may be mediated by the immune suppressive effects of

cocaine/crack use on control of latent human papillomavirus infection (8, 9). Cocaine is known to exert immune suppressive effects, including reduction in delayed-type hypersensitivity responses and reduced effector cell proliferation, in animal and *in vitro* models, providing biological plausibility for this hypothesis (10-13).

Direct assessment of memory T-cell responses in observational cohorts would require HPV type-specific stimulation of tissue-resident lymphocytes, which is not feasible with current technology. Therefore, in order to investigate a possible mediating effect of immune suppression on the association between cocaine/crack use and HPV detection, we evaluated the association between cocaine/crack use and the presence of cutaneous anergy. Cutaneous anergy testing uses common antigens (e.g., *Candida albicans*, tetanus toxoid, and mumps) to assess local T-cell-dependent immune response at the epithelial cell level (14), and has been associated with prevalent cervical lesions and incident oncogenic HPV types in a previous sample of HIV-negative and HIV-positive women in the WIHS (14).

We hypothesize that cocaine use may be associated with suppression of immune function in epithelial tissue, thereby allowing for reactivation of a latent infection. No studies to date have examined the direct association between cocaine use and immune suppression in a U.S. population at high risk for cervical HPV infection and reactivation. Our objective was to assess the association between cocaine use and epithelial immune suppression, measured by cutaneous anergy, in a population of HIV-positive and at-risk HIV-negative women in the United States.

Methods

The Women's Interagency HIV Study (WIHS)

The WIHS is a multicenter prospective cohort study designed to characterize the natural history and pathogenesis of HIV infection and related conditions among a geographically and ethnically diverse population of women. Details of the study have been previously described (15, 16). Briefly, HIV-seropositive and seronegative women were enrolled at six sites in the United States (Bronx/Manhattan, Brooklyn, Chicago, Los Angeles, San Francisco, and Washington, D.C.) over two periods; original recruitment in 1994-1995 included 2,628 women and an additional recruitment was conducted in 2001-2002 when 1,144 women were enrolled. Participants were scheduled for one visit during each 6-month calendar period (April–September and October–March). All participants were followed at visit windows approximately six months apart (plus or minus six weeks), with a median of 11.42 person-years of follow-up for the 1994-1995 recruits and 5.05 person-years for the 2001-2002 recruits. Institutional review board approval was obtained from each local site, and informed consent was obtained for each participant.

At study visits, participants completed an extensive structured interview to collect information on past and current sexual behavior, use of recreational drugs, smoking, alcohol, and medications, and underwent a physical and gynecologic examination. Blood samples were obtained in order to determine HIV serostatus, HIV viral RNA load and CD4 cell counts; CD4+ T cells were measured using flow cytometry in laboratories participating in the AIDS Clinical Trials Quality Assurance Program, and plasma HIV RNA levels were determined using a nucleic acid sequence-based amplification technique (Organon Teknika, Durham, NC) according to the manufacturer's recommended protocol (17).

Cutaneous anergy testing was performed at baseline and every other six-month visit (not exceeding once per year) through March 2000 in the WIHS using the Mantoux technique with 0.1 cc *Candida albicans* antigen (Candin; Allermed Laboratories, Inc., San Diego, California, USA), tetanus toxoid (1:5 dilution of fluid toxoid; Connaught Pharmaceuticals, now Aventis Pasteur, Swiftwater, Pennsylvania, USA), and mumps skin test antigen (Connaught Pharmaceuticals) (14). Test administration and reading were standardized via individual training and by using a training video from the U.S. Centers for Disease Control and Prevention, per WIHS protocol.

Study Population

A total of 1,957 of 3,766 WIHS participants had at least one visit with data on anergy testing, of which 1,520 women had at least one visit in the pre-HAART era (before July 1996). Women who did not have valid anergy test results differed significantly from women who did by the following characteristics ($p < 0.05$ for all characteristics): they were younger, more likely to be Hispanic, more likely to be never married, more likely to reside at a drug treatment facility or someone else's house/apartment, slightly more likely to be employed (but have similar average household income to those who do not have anergy results), more likely to be enrolled at the Los Angeles study site, less likely to have a low-grade cervical lesion, and more likely to be HIV-negative and have HIV viral loads less than 20,000 copies/ml and more likely to be on HAART. Women without valid anergy data were also less likely to have any STIs, more likely to never have smoked cigarettes, and were less likely to report injecting drugs recently, less likely to use cocaine and heroin, but more likely to use

other drugs (including amphetamines, narcotics, and hallucinogens). No differences were found between women with and without anergy data in terms of BMI, alcohol drinking behavior, (illicit) methadone use, and non-injection drug use, including crack and marijuana. After excluding 146 women with history of hysterectomy and 1 woman with missing drug use data, we conducted a cross-sectional analysis on existing, prospectively-collected data on 1,373 women to evaluate the association of drug use and cutaneous anergy prior to the widespread use of HAART (Figure 1) (14).

Outcome Assessment

A participant was considered to have a valid cutaneous anergy test if the test was placed and read between two to three days apart and a measure for all three antigens was recorded (14). Anergy was coded as a dichotomous outcome: non-anergic if induration to any of the three antigens was ≥5 mm and anergic if indurations in response to all three antigens were <5 mm in diameter. This 5mm cutpoint for classifying cutaneous anergy test results is aligned with existing literature describing anergy testing among HIV-positive populations (14, 18-20). Anergic skin testing results indicate a lack of epithelial cell-mediated immunity, or the inability to mount a normal DTH immune response to foreign antigen, and non-anergic skin testing results indicate intact cell-mediated immunity.

Exposure Assessment

Participants' drug use for the prior six-month time period was self-reported during interviews conducted by trained study personnel. Data were collected for the

following drug types: cocaine, crack cocaine, heroin, and marijuana, including frequency of use and route of administration (injection vs. non-injection). For this analysis, any cocaine use includes recent exposure to cocaine or crack cocaine by any route, and will be referred to simply as cocaine use.

Covariates

Age was collected as a continuous variable and re-centered around the mean (35.8 years) for inclusion in multivariate models. Race/ethnicity was categorized as Black, Hispanic, White, and Other. Employment status was dichotomized into currently employed and not currently employed. Highest level of education achieved was categorized as none (no schooling), primary only, some high school, completed high school, some college, completed four years of college, and attended/completed graduate school. Study site was categorized by city or region as Bronx, Brooklyn, Chicago, and District of Columbia/Maryland/Virginia. Measures of sexual risk behaviors included lifetime number of male sexual partners (0-4 partners, 5-9 partners, 10-49 partners, and ≥ 50 partners), number of male sexual partners since last study visit (0, 1, 2, and ≥3 partners), and recent report of sexually transmitted infection (categorized as none or any STIs reported in the previous six months). Data on HIV serostatus (positive, negative), HIV-related immune markers, e.g., CD4+ cell count (>500 cells/mm^3, 200-500 cells/mm^3, <200 cells/mm^3), HIV RNA level (<4,000 copies/ml, 4,000-20,000 copies/ml, 20,000-100,000 copies/ml, ≥100,000 copies/ml), current pregnancy status (pregnant, not pregnant), recent alcohol use (abstainer, light [<3 drinks/week], moderate [3-13 drinks/week], heavy [≥14 drinks/week]), and current smoking status (no, yes)

were also categorized for inclusion in analyses. Body mass index (BMI) was categorized according to standardized national and international guidelines(21) for determining weight status for adults 20 years of age and older: underweight (<18.5), normal weight (18.5-24.9), overweight (25-29.9), and obese (≥30).

Statistical Analysis

Demographic, immunologic, virologic, and behavioral characteristics at the time of anergy measurement were compared across persons who reported any cocaine use compared to those who reported no cocaine use using chi-square and Fisher's exact tests, when necessary. We selected covariates based on *a priori* knowledge and statistically significant associations with exposure and outcome in univariate logistic regression analyses ($p<0.10$). We used a series of logistic regression models to estimate the association between recent use of drugs (any cocaine, any heroin, and any cocaine or heroin in separate models) and anergy adjusting for the following covariates: lifetime and recent number of male sexual partners, use of other drugs (e.g., marijuana and heroin for models with any cocaine exposure, marijuana for models using any cocaine or heroin exposure, cocaine and marijuana for models with any heroin exposure), and BMI. We also stratified on HIV status to examine the potential direct immunological effect of drug use independent of HIV-induced immune suppression.

All statistical tests were two-sided, and a p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using STATA 9.2 (STATACORP, College Station, TX).

Results

Study Population

Demographic characteristics of the study population reflect the overall WIHS cohort from which they were selected(16): 772 (56.2%) were Black, 326 (23.7%) were Hispanic and 236 (17.2%) were White. Median age of women included in the analysis was 35.8 years, with a range of 17-66 years of age (16).

Overall, 286 women (21%) reported recent use (prior six months) of any cocaine or crack cocaine (Table 1). Thirteen percent of the 1,373 women reported recent cocaine use, 15% reported crack use and 11% reported heroin use. Among those who reported any cocaine use, nearly three-quarters reported crack use and 40% reported heroin use. Most women (75%) who reported recent heroin use also used cocaine.

Persons who reported recent cocaine (including crack) use were more likely than those who did not to be older (over 40 years old), Black, unemployed, and have less than a high school education (Table 1). Cocaine users were more likely to be normal weight or underweight, report exchanging sex for money, drugs, or shelter, and report two or more recent male sexual partners and more than fifty lifetime male sexual partners. Women who reported recent cocaine use were more likely to be HIV-negative, have CD4 cell counts greater than 500 cells/mm^3, have lower HIV viral load, and not be on any HIV treatment. Distributions of abnormal Pap smear, cervical treatment since last visit, multiple STIs and pregnancy status did not differ between persons who reported any recent cocaine use and those who did not (Table 1).

Anergy

A total of 635 women (46%) had a diminished DTH response indicating anergy (Table 2). There was no association between cocaine use and anergy in the overall analytic cohort (OR=1.03, 95% C.I.: 0.79-1.34), nor was there any association between use of any individual drugs (e.g., OR for heroin = 0.99, 95% C.I.: 0.71-1.39); OR for marijuana = 0.82, 95% C.I.: 0.61-1.05), OR for crack = 1.08, 95% C.I.: 0.80-1.45), OR for cocaine = 0.84, 95% C.I.: 0.61-1.16) and anergy, or drug administration types (e.g., OR for injection drugs = 0.95, 95% C.I.: 0.65-1.40; OR for non-injection drugs = 0.90, 95% C.I.: 0.72-1.13) and anergy. However, anergy (i.e., diminished DTH responses under 5mm induration) was associated with being older, study site, and being unemployed. Anergic responses were more likely to be observed among women with no recent male sex partners, normal weight, abnormal Pap result, and recently detected oncogenic HPV type. Women with anergy were more likely to be HIV positive and have higher HIV viral load and peripheral CD4+ T-cell counts under 200 cells/mm^3.

Stratified by HIV serostatus

In order to isolate the effect of cocaine use and immune response not influenced by HIV-associated immune suppression, we conducted stratified analysis by HIV serostatus. In unadjusted analysis among HIV uninfected women, we observed a statistically significant, increased odds of anergy associated with recent cocaine use (OR = 1.89, 95% C.I.: 1.05-3.40 (Table 3, Model 1). Adjusting for use of other drugs (e.g., marijuana and heroin) increased the magnitude of the effect estimate by approximately five percent (OR = 1.99, 95% C.I.: 1.03-3.83) (Table 3, Model 2). Adjusting for other potential confounders including age, employment status, recent number of male sex

partners, lifetime number of male sex partners, study site, BMI, and other drug use (marijuana and heroin), the association between recent cocaine use and anergy strengthened in magnitude and remained statistically significant (OR=2.29 (95% C.I.: 1.06-4.95) (Table 3, Model 3).

In unadjusted analysis among HIV uninfected women, we also observed statistically significant, increased odds of anergy among HIV-negative women associated with recent heroin use (OR=2.15; 95% C.I.: 1.06-4.36) (Table 3, Model 1). After adjustment for the aforementioned covariates including crack/cocaine, the effect estimate was attenuated (OR=1.73, 95% C.I.: 0.74-4.04), and results were not statistically significant (Table 3, Model 3).

Among HIV-positive women, there was no evidence for any association between cocaine use and risk of anergy, or for any other drugs and risk of anergy (data not shown).

Discussion

Evidence supporting the biological effect of cocaine use on immune suppression was observed in this study among HIV-uninfected women. There was a greater than two-fold, statistically significant risk of diminished DTH response (indicating anergy) associated with recent cocaine use (including crack) among HIV-uninfected women. By contrast, we did not observe any association between cocaine use and immune suppression among HIV-infected women. Since HIV-infected women have profound HIV-mediated immune suppression, it was unlikely that we would have observed the association of diminished DTH response with cocaine use among participants with HIV

infection. We further did not observe statistically significant associations between other drugs (e.g., heroin) and immune suppression among HIV-infected or -uninfected women.

Among HIV-negative women, our finding that use of cocaine, and not other drugs such as heroin, was independently associated with increased risk of anergy is supported by existing laboratory and clinical data. Cocaine has broad immune suppressive effects in animal and *in vitro* models (11, 22), including decreasing pro-inflammatory IgA+ cells, increasing the number of CD8+ cells in mucosal tissue (23), and inhibiting DTH response to dinitrofluorobenzene (DNFB) (24). Despite a small sample size, an experimental study of 30 healthy men and women demonstrated that cocaine induced suppression of IL-6, a pro-inflammatory cytokine important for mediating host response to infection (25). In contrast to cocaine's direct immunomodulatory effects *in vivo* (12), heroin-associated effects on the immune system are considered indirect and complex, involving interaction with the hypothalamus-pituitary-adrenal (HPA) axis and central nervous system (26, 27). Not surprisingly, we failed to observe independent associations of immune suppression with any other drugs. These differential effects of cocaine and heroin on immune cell function provides further biological support for the association we observed among HIV-uninfected women which appears to be specific to cocaine use rather than global drug use.

Further demonstrating the specific association between cocaine and immune suppression, there was a two-fold risk of anergy with heroin use in an unadjusted model, but after accounting for cocaine use, the estimate was attenuated and lost statistical significance (Table 3, Model 3, Heroin). Based on the independent associations

observed with cocaine and that the majority of heroin users in this study also reported using cocaine, it is possible that the association between any cocaine or heroin and anergy may be largely driven by cocaine use (Table 3, Model 3, Any cocaine or heroin).

In addition, epidemiologic evidence from this cohort (9) and a similar HIV-positive and at-risk HIV-negative U.S. cohort (8) shows similar specificity of association between cocaine use and HPV infection. We observed an increase in effect size after accounting for sexual risk behaviors in this study, which may suggest that a cocaine-HPV association may be mediated by the immune suppressive effects of cocaine use on control of latent (undetectable) HPV infection, further lending support for a direct biological cause and potentially lesser influence of sexual risk on new HPV detection.

This study has several potential limitations. We did not have sufficient power to examine the effect of route of administration of drug use, frequency of use, nor cumulative drug use. However, we used existing data from a nationally-representative sample of at-risk, HIV-uninfected women in the United States and were able to identify an independent cocaine association with cutaneous anergy. In addition to limited statistical power, there are inherent difficulties in the clinical measure used to test for anergy. First, skin testing results are dependent on recall antigens. If an individual had not been exposed to the antigen prior to the placement of recall antigen, there would be no visible induration mounted in response to a novel antigen, thus appearing anergic without truly having reduced cell-mediated immunity. Nevertheless, if this occurred, we would expect this non-differential misclassification to bias our results towards the null. Secondly, exposure to common antigens may vary geographically (18); however, it is

accepted that the three antigens used in this study are equally distributed throughout the United States (28). To safeguard against bias from differential antigen exposures across study sites, we included a covariate for study site in multivariate models to account for potential antigenic variation as well as potential variability in drug formulation and potency across sites. Other investigators have suggested that anergy may be best classified using simply two antigens: mumps and tetanus (18). However, anergy testing has been typically and historically defined in the clinic and in other epidemiologic studies as any induration observed to the three antigens we used in our analysis: tetanus, mumps, and Candida antigens (14, 28, 29). Sensitivity analyses using a two antigen definition (mumps and tetanus) did not yield results that differed substantially from what we report here.

A recent review of HPV clinical immunology (30) highlighted that immune evasion by HPV uses strategies of downregulation of the innate immune system via Langerhans' cells, dendritic cells, and toll-like receptors (TLRs), and of the cell-mediated immune system's T cells and neutralizing antibodies. We acknowledge that other immune markers, such as diminished plasmacytoid dendritic cell (PDC) counts, which have been found to be predictive of infections among HIV-infected women (30), or gamma interferon and interleukin-2 cytokines, which have been described as optimal *in vitro* correlates of DTH (31), may be more direct measures of drug-induced immune suppression. Markers such as these may be important for control of HPV infection and reactivation, and may be explored in future studies as potential indicators of drug-associated immune compromise. However, anergy skin testing is an easily administered

and well-tolerated method to measure cell-mediated immunity at the cutaneous surface (31).

Despite these potential limitations, there were several strengths to this study. Using a nationally representative sample of women who are at risk for HIV infection and opportunistic infections, we were able to account for use of other drugs including heroin and marijuana in the same time period as our main exposure of interest, cocaine. Polysubstance abuse is common among drug-using populations (32), and it is critical to consider the potential combined effects, whether antagonistic or synergistic, of multiple drug use. By creating exposure variables based on the chemical compound(s) used, regardless of route of administration, we were able to identify striking effects with exposure to any cocaine formulation (crack cocaine or cocaine), while effects of heroin exposure on this type of immunologic response were not supported by the data. To our knowledge, this study is the first to examine the direct effect of cocaine use on cutaneous anergy in the context of immune suppression and HPV infection in high-risk, drug-using women.

In summary, cocaine use was associated with a marker of epithelial immune suppression, and supports the hypothesis that the association of cocaine with new HPV detection might be explained by loss of immunologic control, as well as new acquisition. Anergy skin testing serves as a simple clinical measure of lack of immune response at the epithelial cell level, which may be analogous to the immune microenvironment responsible for suppressing active HPV replication at the cervix, further supporting the hypothesis that cocaine-using women are susceptible to reactivation of latent HPV infection. These findings call attention to careful evaluation of risk factors for new HPV

detection in the context of risk of loss of immunologic control of undetectable persistent (latent) infections, as well as risk of acquisition. As the burden of drug use among women has increased since 2000 (33), it is crucial to understand potential implications of drug use on immunologic states favoring HPV proliferation in these high-risk and vulnerable populations.

References

1. CDC. Genital HPV Infection - Fact Sheet. In: Centers for Disease Control and Prevention, National Center for HIV/AIDS, Viral Hepatits, STD, and TB Prevention, Division of STD Prevention; 2013.
2. Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, et al. Prevalence of HPV infection among females in the United States. Jama 2007;297(8):813-9.
3. Brown DR, Shew ML, Qadadri B, Neptune N, Vargas M, Tu W, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis 2005;191(2):182-92.
4. Stanley M. Immune responses to human papillomavirus. Vaccine 2006;24 Suppl 1:S16-22.
5. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract. Infect Dis Obstet Gynecol 2006;2006 Suppl:40470.
6. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest 2011;121(12):4593-9.
7. Maglennon GA, McIntosh P, Doorbar J. Persistence of viral DNA in the epithelial basal layer suggests a model for papillomavirus latency following immune regression. Virology 2011;414(2):153-63.
8. Phelan DF, Gange SJ, Ahdieh-Grant L, Mehta SH, Kirk GD, Shah K, et al. Determinants of Newly Detected Human Papillomavirus Infection in HIV-Infected and HIV-Uninfected Injection Drug Using Women. Sex Transm Dis 2009.
9. Minkoff H, Zhong Y, Strickler HD, Watts DH, Palefsky JM, Levine AM, et al. The relationship between cocaine use and human papillomavirus infections in HIV-seropositive and HIV-seronegative women. Infect Dis Obstet Gynecol 2008;2008:587082.
10. Friedman H, Newton C, Klein TW. Microbial infections, immunomodulation, and drugs of abuse. Clin Microbiol Rev 2003;16(2):209-19.
11. Baldwin GC, Roth MD, Tashkin DP. Acute and chronic effects of cocaine on the immune system and the possible link to AIDS. J Neuroimmunol 1998;83(1-2):133-8.
12. Donahoe RM, Falek A, Madden JJ, Nicholson JK, Bokos P, Gallegos K, et al. Effects of cocaine and other drugs of abuse on immune function. Adv Exp Med Biol 1991;288:143-50.
13. Friedman H, Pross S, Klein TW. Addictive drugs and their relationship with infectious diseases. FEMS Immunol Med Microbiol 2006;47(3):330-42.
14. Harris TG, Burk RD, Xue X, Anastos K, Minkoff H, Massad LS, et al. Association of cutaneous anergy with human papillomavirus and cervical neoplasia in HIV-seropositive and seronegative women. Aids 2007;21(14):1933-41.
15. Bacon MC, von Wyl V, Alden C, Sharp G, Robison E, Hessol N, et al. The Women's Interagency HIV Study: an observational cohort brings clinical sciences to the bench. Clin Diagn Lab Immunol 2005;12(9):1013-9.
16. Barkan SE, Melnick SL, Preston-Martin S, Weber K, Kalish LA, Miotti P, et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. Epidemiology 1998;9(2):117-25.

17. Ahdieh-Grant L, Li R, Levine AM, Massad LS, Strickler HD, Minkoff H, et al. Highly active antiretroviral therapy and cervical squamous intraepithelial lesions in human immunodeficiency virus-positive women. J Natl Cancer Inst 2004;96(14):1070-6.
18. Klein RS, Flanigan T, Schuman P, Smith D, Vlahov D. Criteria for assessing cutaneous anergy in women with or at risk for HIV infection. HIV Epidemiologic Research Study Group. J Allergy Clin Immunol 1999;103(1 Pt 1):93-8.
19. Rogers AS, Ellenberg JH, Douglas SD, Henry-Reid L, Peralta L, Wilson CM. Performance of antigens used in detecting delayed-type hypersensitivity in adolescents infected with the human immunodeficiency virus. Clin Diagn Lab Immunol 2001;8(2):273-8.
20. Anergy skin testing and tuberculosis [corrected] preventive therapy for HIV-infected persons: revised recommendations. Centers for Disease Control and Prevention. MMWR Recomm Rep 1997;46(RR-15):1-10.
21. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1995;854:1-452.
22. Ou DW, Shen ML, Luo YD. Effects of cocaine on the immune system of Balb/C mice. Clin Immunol Immunopathol 1989;52(2):305-12.
23. Lopez MC, Watson RR. Effect of cocaine and murine AIDS on lamina propria T and B cells in normal mice. Life Sci 1994;54(9):PL147-51.
24. Watson ES, Murphy JC, ElSohly HN, ElSohly MA, Turner CE. Effects of the administration of coca alkaloids on the primary immune responses of mice: interaction with delta 9-tetrahydrocannabinol and ethanol. Toxicol Appl Pharmacol 1983;71(1):1-13.
25. Halpern JH, Sholar MB, Glowacki J, Mello NK, Mendelson JH, Siegel AJ. Diminished interleukin-6 response to proinflammatory challenge in men and women after intravenous cocaine administration. J Clin Endocrinol Metab 2003;88(3):1188-93.
26. McCarthy L, Wetzel M, Sliker JK, Eisenstein TK, Rogers TJ. Opioids, opioid receptors, and the immune response. Drug Alcohol Depend 2001;62(2):111-23.
27. Alonzo NC, Bayer BM. Opioids, immunology, and host defenses of intravenous drug abusers. Infect Dis Clin North Am 2002;16(3):553-69.
28. Wright PW, Crutcher JE, Holiday DB. Selection of skin test antigens to evaluate PPD anergy. J Fam Pract 1995;41(1):59-64.
29. Mofenson LM, Rodriguez EM, Hershow R, Fox HE, Landesman S, Tuomala R, et al. Mycobacterium tuberculosis infection in pregnant and nonpregnant women infected with HIV in the Women and Infants Transmission Study. Arch Intern Med 1995;155(10):1066-72.
30. Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T, et al. Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis 2009;9(6):347-56.
31. Rosenstreich DL. Evaluation of delayed hypersensitivity: from PPD to poison ivy. Allergy Proc 1993;14(6):395-400.
32. Leri F, Bruneau J, Stewart J. Understanding polydrug use: review of heroin and cocaine co-use. Addiction 2003;98(1):7-22.
33. Kay A, Taylor TE, Barthwell AG, Wichelecki J, Leopold V. Substance use and women's health. J Addict Dis 2010;29(2):139-63.

Table 1. Demographic, clinical, and behavioral characteristics by any recent cocaine use (including crack cocaine) reported at cutaneous anergy test visit, Women's Interagency HIV Study (WIHS), 1994-1996

Characteristic	No cocaine use N	%	Any cocaine use N	%	Total N	p-value*
Total	1087	79.2	286	20.8	1373	
Demographics						
Age (years)						
<25	92	8.5	6	2.1	98	<.0005
25-29	181	16.7	41	14.3	222	
30-34	260	23.9	65	22.7	325	
35-39	294	27.1	81	28.3	375	
40-44	1557	14.4	63	22.0	220	
≥45	103	9.5	30	10.5	133	
Race						
Black	582	53.5	190	66.4	772	0.001
Hispanic	275	25.3	51	17.8	326	
Other	36	3.3	3	1.1	39	
White	194	17.9	42	14.7	236	
Study site						
Bronx	282	25.9	103	36.0	385	<.0005
Brooklyn	241	22.2	42	14.7	283	
Chicago	151	13.9	47	16.4	198	
DC/MD/VA	169	15.6	22	7.7	191	
LA	141	13.0	22	7.7	163	
SF	103	9.5	50	17.5	153	
Employment status						
Not employed	759	69.8	258	90.2	1017	<.0005
Employed	325	29.9	28	9.8	353	
Highest level of education achieved						
Less than high school	367	33.8	128	44.8	495	0.001
Completed high school	720	66.2	158	55.2	878	

Characteristic	No cocaine use N	%	Any cocaine use N	%	Total N	p-value
Substance Use						
Cocaine use						
No	1087	100	110	38.5	1197	<.0005
Yes	0	0	176	61.5	176	
Crack cocaine use						
No	1087	100	75	26.2	1162	<.0005
Yes	0	0	211	73.8	211	
Heroin use						
No	1048	96.4	171	59.8	1219	<.0005
Yes	39	3.6	115	40.2	154	
Marijuana use						
No	892	82.2	139	48.6	1031	<.0005
Yes	193	17.8	147	51.4	340	
Injection drug use						
No	1062	97.7	198	69.2	1260	<.0005
Yes	25	2.3	88	30.8	113	
Non-injection drug use						
No	887	81.6	32	11.2	919	<.0005
Yes	200	18.4	254	88.8	454	
Current cigarette smoking						
No	563	51.8	33	11.5	596	<.0005
Yes	524	48.2	253	88.5	777	
Alcohol use (drinks/week)						
Abstainer	541	49.8	63	22.0	604	<.0005
Light (<3)	343	31.6	75	26.2	418	
Moderate (3-13)	138	12.7	73	25.5	211	
Heavy (≥14)	42	3.9	71	24.8	113	
Sexual Risk Behavior						
Exchanged sex for money, drugs, or shelter						
No	735	67.6	118	41.3	853	<.0005
Yes	348	32.0	167	58.4	515	

Characteristic	No cocaine use N	%	Any cocaine use N	%	Total N	p-value
Male sex partners						
None	337	31.0	69	24.1	406	<.0005
1	621	57.1	119	41.6	740	
2	88	8.1	41	14.3	129	
3 or more	39	3.6	55	19.2	94	
Lifetime male sex partners						
0-4	206	19.0	28	9.8	234	<.0005
5-9	246	22.6	43	15.0	289	
10-49	357	32.8	88	30.8	445	
≥50	244	22.5	114	39.9	358	
HIV-Related Characteristics						
HIV serostatus						
Converter identified at death	4	0.4	5	1.8	9	<.0005
Negative	197	18.1	75	26.2	272	
Prevalent (positive)	886	81.5	206	72.0	1092	
HIV viral load (copies/ml)						
< 4,000	113	10.4	37	12.9	150	0.001
4,000-20,000	350	32.2	74	25.9	424	
20,000-100,000	191	17.6	55	19.2	246	
>100,000	209	19.2	36	12.6	245	
CD4 cell count (cells/mm^3)						
> 500	398	36.6	130	45.5%	528	0.004
200-500	398	36.6	98	34.3%	496	
< 200	262	24.1	46	16.1%	308	
Clinical Characteristics						
Body mass index (BMI; kg/m^2)						
Underweight, <18.5	30	2.8	18	6.5	48	<.0005
Normal weight, 18.5-24.9	405	37.9	151	54.1	556	
Overweight, 25-29.9	320	29.9	73	26.2	393	
Obese, ≥30	314	29.4	37	13.3	351	

Characteristic	No cocaine use N	%	Any cocaine use N	%	Total N	p-value
Anergy skin test result						
Non-anergic	586	53.9	152	53.1	738	0.82
Anergic	501	46.1	134	46.9	635	
Abnormal Pap result						
No	909	83.6	229	80.1	1138	0.28
Yes	139	12.8	47	16.4	186	
HPV type(s) detected						
Non-oncogenic HPV	308	28.3	72	25.2	380	0.003
Any Oncogenic HPV	273	25.1	81	28.3	354	
No HPV (Negative)	459	42.2	106	37.1	565	
Current pregnancy status						
No	1062	97.7	279	97.6	1341	0.46
Yes	17	1.6	3	1.1	20	

*p-values reported are based on chi-squared or Fisher's exact tests

Column percentages may not sum to 100% due to rounding and/or missing values.

Characteristics refer to the prior six month period unless otherwise noted.

Abbreviations: STI, sexually transmitted infection; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy; HPV, human papillomavirus

Table 2. Demographic, clinical, and behavioral characteristics reported at cutaneous anergy test visit by presence of cutaneous anergy, Women's Interagency HIV Study (WIHS), 1994-1996

Characteristic	Non-anergic N	%	Anergic N	%	Total N	p-value*
Total	738	53.7	635	46.3	1373	
Demographics						
Age (years)						
<25	61	8.3	37	5.8	98	0.02
25-29	136	18.4	86	13.5	222	
30-34	179	24.3	146	23.0	325	
35-39	184	24.9	191	30.1	375	
40-44	111	15.0	109	17.2	220	
≥45	67	9.1	66	10.4	133	
Race						
Black	429	58.1	343	54.0	772	0.42
Hispanic	170	23.0	156	24.6	326	
Other	18	2.4	21	3.3	39	
White	121	16.4	115	18.1	236	
Study site						
Bronx	194	26.3	191	30.1	385	<0.0005
Brooklyn	180	24.4	103	16.2	283	
Chicago	119	16.1	79	12.4	198	
DC/MD/VA	98	13.3	93	14.7	191	
LA	68	9.2	95	15.0	163	
SF	79	10.7	74	11.7	153	
Employment status						
Not employed	522	70.7	495	78.0	1017	0.006
Employed	215	29.1	138	21.7	353	
Highest level of education achieved						
Less than high school	272	36.9	223	35.1	495	0.50
Completed high school	466	63.1	412	64.9	878	

Characteristic		Non-anergic N	%	Anergic N	%	Total N	p-value
Substance Use							
Cocaine, crack cocaine, or heroin							
	No	565	76.6	483	76.1	1048	0.83
	Yes	173	23.4	152	23.9	325	
Any cocaine (including crack)							
	No	586	79.4	501	78.9	1087	0.82
	Yes	152	20.6	134	21.1	286	
Any heroin							
	No	655	88.7	564	88.8	1219	0.97
	Yes	83	11.3	71	11.2	154	
Crack cocaine							
	No	628	85.1	534	84.1	1162	0.61
	Yes	110	14.9	101	15.9	211	
Marijuana							
	No	541	73.3	490	77.2	1031	0.12
	Yes	195	26.4	145	22.8	340	
Injection drug							
	No	676	91.6	584	92.0	1260	0.81
	Yes	62	8.4	51	8.0	113	
Non-injection drug							
	No	486	65.9	433	68.2	919	0.36
	Yes	252	34.1	202	31.8	454	
Current cigarette smoking							
	No	322	43.6	274	43.2	596	0.86
	Yes	416	56.4	361	56.9	777	
Alcohol Use (drinks/week)							
	Abstainer	316	42.8	288	45.4	604	0.65
	Light (<3)	227	30.8	191	30.1	418	
	Moderate (3-13)	120	16.3	91	14.3	211	
	Heavy (≥14)	63	8.5	50	7.9	113	

Characteristic	Non-anergic N	%	Anergic N	%	Total N	p-value
Sexual Risk Behavior						
Exchanged sex for money, drugs, or shelter						
No	454	61.5	399	62.8	853	0.71
Yes	282	38.2	233	36.7	515	
Male sex partners						
None	190	25.8	216	34.0	406	0.007
1	412	55.8	328	51.7	740	
2	81	11.0	48	7.6	129	
3 or more	52	7.1	42	6.6	94	
Lifetime number of male sexual partners						
0-4	114	15.4	120	18.9	234	0.45
5-9	159	21.5	130	20.5	289	
10-49	250	33.9	195	30.7	445	
≥50	190	25.8	168	26.5	358	
HIV-Related Characteristics						
HIV serostatus						
Converter identified at death	6	0.8	3	0.5	9	<0.0005
Negative	211	28.6	61	9.6	272	
Prevalent (positive)	521	70.6	571	89.9	1092	
HIV viral load (copies/ml)						
< 4,000	93	12.6	57	9.0	150	<0.0005
4,000-20,000	252	34.2	172	27.1	424	
20,000-100,000	111	15.0	135	21.3	246	
>100,000	52	7.1	193	30.4	245	
CD4 cell count (cells/mm^3)						
> 500	393	53.3	135	21.3	528	<0.0005
200-500	267	36.2	229	36.1	496	
< 200	61	8.3	247	38.9	308	

Characteristic	Non-anergic N	%	Anergic N	%	Total N	p-value
Clinical Characteristics						
Body mass index (BMI; kg/m^2)						
Underweight, <18.5	26	3.5	22	3.5	48	0.07
Normal weight, 18.5-24.9	273	37.0	283	44.6	556	
Overweight, 25-29.9	221	30.0	172	27.1	393	
Obese, ≥30	204	27.6	147	23.2	351	
Abnormal Pap smear result						
No	659	89.3	479	75.4	1138	<0.0005
Yes	54	7.3	132	20.8	186	
HPV Type(s) detected						
Non-oncogenic HPV	206	27.9	174	27.4	380	<0.0005
Any Oncogenic HPV	140	19.0	214	33.7	354	
No HPV (Negative)	348	47.2	217	34.2	565	
Current pregnancy status						
No	721	97.7	620	97.6	1341	0.90
Yes	10	1.4	10	1.6	20	

* p-values are based on chi-squared or Fisher's exact tests.

Note: Non-anergic defined by induration ≥5mm, anergic defined by induration >5mm.

Column percentages may not sum to 100% due to rounding and/or missing values.

Characteristics refer to the prior six month period unless otherwise noted.

Abbreviations: STI, sexually transmitted infection; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy; HPV, human papillomavirus

Table 3. Odds ratio (OR) estimates from unadjusted and adjusted logistic regression models of cutaneous anergy testing results, pre-HAART period 1994-1996, Women's Interagency HIV Study (WIHS) (N=1,373)

Reported drug use (prior six months)	N	Model 1: Unadjusted OR (95% C.I.)	N	Model 2: Adjusted OR[a] (95% C.I.)	N	Model 3: Adjusted OR[b] (95% C.I.)
Overall cohort						
Any cocaine (including crack) or heroin						
≥5mm induration	738	Ref	736	Ref	696	Ref
<5mm induration	635	1.03 (0.80-1.32)	635	1.12 (0.86-1.45)	602	0.99 (0.73-1.33)
Any cocaine (including crack)						
≥5mm induration	738	Ref	737	Ref	697	Ref
<5mm induration	635	1.03 (0.80-1.34)	635	1.19 (0.88-1.59)	602	1.08 (0.78-1.50)
Heroin						
≥5mm induration	738	Ref	736	Ref	696	Ref
<5mm induration	635	0.99 (0.71-1.39)	635	1.07 (0.74-1.53)	602	0.91 (0.61-1.35)
HIV-negative women						
Any cocaine (including crack) or heroin						
≥5mm induration	217	Ref	217	Ref	205	Ref
<5mm induration	64	2.16 (1.22-3.82)[c]	64	2.52 (1.37-4.65)[d]	63	2.85 (1.35-6.04)[c]
Any cocaine (including crack)						
≥5mm induration	217	Ref	217	Ref	205	Ref
<5mm induration	64	1.89 (1.05-3.40)[e]	64	1.99 (1.03-3.83)[e]	63	2.29 (1.06-4.95)[e]
Heroin						
≥5mm induration	217	Ref	217	Ref	205	Ref
<5mm induration	64	2.15 (1.06-4.36)[e]	64	2.03 (0.97-4.28)	63	1.73 (0.74-4.04)

[a] Model 2: Adjusted for other drug use only (e.g., adjusted for any recent marijuana use only for cocaine/crack/heroin exposure; adjusted for recent marijuana and/or heroin use

for cocaine/crack exposure; adjusted for recent crack, cocaine and/or marijuana use for heroin exposure)
[b] Model 3: Adjusted for other drug use, age (centered), employment status, recent number of male sex partners, lifetime number of male sex partners, study site, and body mass index (BMI)
[c] p-value < 0.01
[d] p-value < 0.005
[e] p-value < 0.05
Anergy is indicated by <5mm induration.

Chapter Three

Cocaine use and incident HPV- and non-HPV-associated cervical cytological abnormalities among injection drug using women in Baltimore, Maryland

Abstract

Background: Cocaine use has been observed to be associated with acquisition of HPV infection and prevalent cervical cancer, but few studies have focused primarily on the impact of cocaine use on cervical cytological abnormalities.

Objective: We sought to evaluate the association between use of crack/cocaine and risk of incident cervical cytological abnormalities and to quantify the relative burden of cytological abnormalities among current and former injection drug-using women in Baltimore, MD.

Methods: We conducted a longitudinal analysis of 177 HIV-infected and 89 HIV-uninfected female participants enrolled in a18-year prospective community-based cohort study of HIV natural history. The women contributed 1,864 visits where valid Pap testing results were available. To estimate the association between recent use of cocaine and risk of incident cytological abnormality, we conducted logistic regression using generalized estimating equations and conducted stratified analysis by HIV serostatus.

Results: Recent crack/cocaine use was associated with a slight, non-statistically significant increased risk of incident cervical cytological abnormality among HIV-uninfected women (OR 1.29, 95% C.I.: 0.65-2.57). No association was observed among HIV-infected women (OR 1.01, 95% C.I.: 0.69-1.48).

Conclusion: Use of crack/cocaine among HIV-uninfected female injection drug users was marginally associated with risk of incident cervical cytological abnormalities. Cervical cytological abnormalities detected by Pap smear may reflect cellular changes not associated with risk of HPV-associated cervical cancer. Further examination to clarify the independent associations of cocaine use with HPV-associated clinical outcomes will require improved detection and larger populations of HIV-uninfected drug-using women.

Introduction

In 2012, cocaine was the third-most commonly used illicit drug in the United States, with the highest rate of past year dependence or abuse (after marijuana and pain relievers) in the United States, translating to 1.65 million persons with cocaine dependence or abuse out of a total of 23.9 million persons aged 12 or older classified with illicit drug dependence or abuse (1). Rates of current cocaine use among women have remained relatively stable in recent years; 0.3% of females reported using cocaine in the past month in 2012 (1).

Female drug users encounter multiple risks associated with drug use, including social and medical health risks such as drug overdose and HIV (2). Use of illicit drugs, specifically, cocaine, has been linked to acquisition of HPV infection(3, 4) and prevalent invasive cervical cancer(5) in previous epidemiologic studies. Previously in an urban community-based cohort of current and former injection drug users (IDUs), prevalent and incident cervical lesions were found to be associated with crack/cocaine use, but the findings were limited to a small subset of the overall cohort that was part of a five-year gynecological sub-study focusing on HPV risk.(3) To date, few studies have examined the association between cocaine use and HPV-associated clinical outcomes.(3, 4, 6)

Using over 18 years of follow-up data in this same community-based cohort of current and former IDUs in Baltimore, Maryland, we aimed to further explore the risk of incident cytological abnormalities among both HIV-infected and HIV-uninfected women who use crack/cocaine, and to quantify the relative burden of cervical cytological abnormalities in drug-using populations in order to better understand the role of crack/cocaine use in the natural history of HPV infection.

Methods

Study Population

A total of 391 women followed in the AIDS Linked to the IntraVenous Experience (ALIVE) study contributed 3,995 visits with any Pap test data to this analysis. The ALIVE study is a prospective cohort study of male and female current and former injection drug users (IDUs) in Baltimore, Maryland with initial enrollment in 1988-1989, and subsequent rounds of recruitment in 1994-1995, 1998, 2000 and 2005-2008. Details of the study have been previously described.(7) In brief, 2,942 IDUs were initially recruited in 1988-1989 and followed semi-annually. At the following enrollment periods, a total of 1,603 participants were enrolled. At baseline, the majority of ALIVE participants were African-American (94%) and 20% were female. Baseline and semiannual follow-up visits included a standardized interviewer-administered (pre-1998) or audio computer-assisted self-interview (ACASI) (post-1998) questionnaire to ascertain information on demographic characteristics, drug injection and non-injection behaviors, medical history, and sexual and drug-related risk behaviors. Additionally, all HIV positive participants and a random sample of HIV negatives (every fifth seropositive participant triggered the next enrolled seronegative participant to be invited) were invited to participate in the clinical immunologic follow-up including a physical examination and testing for immunologic markers. Women also underwent Papanicolau (Pap) smears and pelvic examinations, as well as cervico-vaginal lavage to collect cervical specimens at semiannual follow-up visits. Pap smears were collected first, with a spatula, followed by a cytobrush (except in pregnancy, where a cotton swab was used). Pap smears were stained and screened by a qualified cytotechnologist by standard criteria for cytopathologic diagnosis. All Pap smears were read by an external, commercial lab (Quest Diagnostics). The institutional review board at the Johns Hopkins University reviewed and approved the study procedures. All study participants provided written informed consent before enrollment in the study.

Female participants without a history of cervical cancer who had at least two valid Pap smear results during follow-up and complete data on crack/cocaine exposure at the visit where the Pap smear was taken were considered eligible for inclusion in this analysis. Women whose Pap

result indicated cervical cancer (n=2) at the first study visit were also excluded, since having cancer would preclude these women from being at-risk for incident cytological abnormality. Nine additional women had cervical cancer over the course of follow-up; we excluded the observations when cervical cancer was first reported and subsequent visits for these women (n=27 visits). Overall, women whose visits were excluded were more likely to be more immune suppressed (CD4 cell counts less than 200) (p<.0001) and less likely to report recent sexual intercourse with an injection drug user (p=0.016). However, women who were excluded were similar to eligible women in terms of age, BMI, cigarette smoking, sharing injection equipment, and recent sexual risk behavior including sexual intercourse with an anonymous partner and number of recent male sexual partners. The resulting analytic cohort included 277 women who contributed a total of 1,864 visits. The overall number of observations included per woman ranged from one to 30, with a median of five visits per participant.

Statistical analysis

Our goal was to determine if crack/cocaine use was associated with risk of incident abnormal Pap results, including atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), and high-grade SIL (HSIL) as classified by the Bethesda System(8). The outcome in this study was ASCUS or more severe cytological abnormality detected by Pap smear at a follow-up visit (henceforth termed the 'index visit', *i*) that was not detected at the immediately preceding visit (*i*-1) with less than seven months between the two consecutive visits. We employed a cut-off of seven months between consecutive visits in order to minimize misclassification of the outcome and time-varying covariates, as these data were collected at each study visit referencing the prior six month time period. Visits that were excluded (53%) were significantly more likely to be among women who were younger (p=<.0001), underweight (p<.0001), HIV-infected (p<.0001), and reported greater than two recent male sex partners (p=0.03), but did not differ from visits that were included by women's drug use status or other measures of sexual risk.

We classified a woman as 'exposed' at a visit if she reported recent (during the prior six months) use of cocaine in any form and by any route, whether cocaine was injected, snorted, smoked (in the form of crack cocaine), or included as part of 'speedball' (combination heroin and cocaine use). We did not differentiate by route or formulation of drug as our primary interest was to examine the influence of the chemical compound of cocaine on cytological abnormalities. Henceforth, we will refer to the exposure as 'crack/cocaine'.

We analyzed frequency tables comparing index visit characteristics of women who reported recent crack/cocaine use to women who did not report any crack/cocaine use, using Pearson's chi-square and Fisher's exact tests to compare differences in distributions of categorical variables (including recent use of injection and non-injection drugs, marijuana and heroin, sharing needles, HIV status, race, cigarette smoking, and various indicators of sexual risk behavior such as trading sex for money or drugs, sex with anonymous partners, STIs, and recent numbers of male sex partners), and Student's t-tests and Wilcoxon rank-sum test to compare distributions of continuous variables (including total times injected in the last six months and last 30 days).(Table 1) Due to the known correlation among measures taken on the same individuals over time, we examined the fit of various covariance patterns using the quasi-likelihood information criterion (QIC) for non-likelihood based methods such as generalized estimating equations (GEE)(9) and selected the exchangeable (uniform) correlation structure to model the covariance among repeated measures within individual women in this unequally-spaced, longitudinal dataset. To analyze the effect of self-reported cocaine use on incident cytological abnormalities over time, we used logistic regression models with GEE, which accounts for the correlation within subjects' repeated measures over time by modeling the covariance structure within subjects.(10)

A subset of women (n=190) between September 1992 and September 1997 had HPV type detection performed on specimens obtained from cervicovaginal lavage (CVL)(methods described elsewhere(3)). Polymerase chain reaction (PCR) amplification of HPV genomic sequences using consensus primers was performed on CVL specimens to detect oncogenic and

non-oncogenic HPV types (including HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 66, 68, 73, 82, 83, 84), with negative and beta-globin controls used to ensure validity of HPV test results(11). Because standard of care in the United States is to triage an ASCUS positive test with HR-HPV DNA testing, we conducted a sensitivity analysis on 190 women with available concurrent visit HPV type and Pap results to examine the possible influence of HPV negative ASCUS positive results on our analysis. Outcomes in this logistic regression analysis using GEE were defined by HR-HPV positive abnormal Pap result (ASCUS, LSIL, or HSIL) only.

Covariates that were found to be statistically significant at a level $p<0.10$ in univariate logistic regression models, as well as those selected based on *a priori* knowledge, were included as potential confounders in the final model, including: age (<35 years; 35-49 years; 40-45 years; >45 years), recent cigarette smoking (yes/no), body mass index (BMI) category (underweight <18.5 kg/m^2; normal weight 18.5-24.9 kg/m^2; overweight 25-29.9 kg/m^2; obese ≥30 kg/m^2) and self-reported history of STIs (any/none). In order to separate the independent effects of crack/cocaine use from the known effects of HIV-related immune suppression on incident HPV-associated abnormal Pap results(12), we conducted stratified analysis by HIV serostatus where possible. Data preparation was conducted in SAS, version 9.2 (SAS Institute Inc., Cary, North Carolina) and longitudinal analyses were conducted in STATA, version 9.2 (StataCorp, College Station, Texas).

Results

Description of Study Population

The median age of the 277 women at the index visit was 39.3 years and 94% of women were African-American (Table 1). The majority of women were HIV-positive at the index visit (68%), with similar prevalence of HIV positivity among non-crack/cocaine users and crack/cocaine users. Women who reported using crack/cocaine at the index visit were more likely to be underweight (BMI < 18.5 kg/m^2) and normal weight (18.5-24.9 kg/m^2) compared to those

who did not. Sexual risk behaviors reported at the index visit differed significantly between women who reported recent crack/cocaine use and women who did not; crack/cocaine users were more likely to report recent sexual intercourse with an IDU, recent sexual intercourse with an anonymous partner, and greater than three male sexual partners in the previous six months compared to those who reported no crack/cocaine use (Table 1). Compared to women who did not report recent crack/cocaine use, women who did were more likely to report recent injection and non-injection drug use, and using heroin, marijuana, and sharing injection equipment (Table 1). Combination injection cocaine and heroin use, i.e. 'speedball', was reported by almost two-thirds (66%) of crack/cocaine users.

Crack/cocaine use and incident cervical cytological abnormalities

The majority (59%) of women reported recent use of crack/cocaine at the index visit. At the index visit, 11% of women who did not report any crack/cocaine use and 15% of those who reported crack/cocaine use had Pap results that indicated any incident cervical epithelial abnormality (either ASCUS or low- or high- grade SIL), but the difference was not statistically-significant (Table 1a).

Table 2 shows the results of longitudinal analyses examining the association between recent crack/cocaine and incident cervical cytological abnormality. In an unadjusted logistic regression model with GEE, recent crack/cocaine use was associated with a slight increased risk of incident cytological abnormality in the overall cohort, but the estimate was not statistically significant (OR 1.23, 95% C.I.: 0.93-1.63).

In univariate logistic regression analysis using GEE, younger age, lower BMI, self-reported history of STIs, and cigarette smoking were found to be associated with both crack/cocaine exposure and incident cytological abnormality; therefore, they were included in the multivariate model as covariates. When added to the final multivariate models, other measures of sexual risk behavior, including recent number of male sexual partners and recent intercourse with an IDU or anonymous partner, did not substantially alter our results.

After stratification by HIV serostatus, we observed a positive association between cocaine use and incident abnormalities among HIV-uninfected women in an unadjusted model, but the estimate did not achieve statistical significance (OR=1.78, 95% C.I.: 0.95-3.34). This association was attenuated after adjusting for age, self-reported history of STIs, cigarette smoking, and BMI) (OR=1.29, 95% C.I.: 0.65-2.57). No association was observed in HIV-infected women in univariate analysis (OR=1.09, 95% CI: 0.77-1.54) or after adjustment for age, self-reported history of STIs, cigarette smoking, and BMI (OR=1.01, 95% C.I.: 0.69-1.48) as well as after additional adjustment for ART use and CD4 cell count (OR=1.03, 95% C.I.: 0.70-1.53).

Crack/cocaine use and incident HPV-positive cervical cytological abnormalities

Because an HR-HPV negative/ASCUS positive test is not considered to be a marker for intraepithelial neoplasia (13), we conducted a sensitivity analysis among the subset of women who had participated in a gynecological sub-study (details described elsewhere(3)) which included HPV genotyping. Data on HPV status and incident Pap results were available for 625 visits among 190 women, or about 34% of the visits included in the main analysis contributed by 69% of the women in the main analysis. The majority of women whose Pap results indicated incident cytological abnormality were also HPV positive: 49 of 68 visits with any incident Pap abnormality indicated presence of concurrent HPV infection (72%). However, 19 of 68 incident cytological abnormalities (28%) were HPV negative.

For this sub-analysis, one hundred-ninety women had eligible visits and contributed a median of three visits each, with a range from one to nine visits. When re-defining the outcome as incident cytological abnormality with concurrent HPV positivity, women with incident HPV-positive cytological abnormality tended to be younger (p=.026), more likely to report a history of STIs (p=.045), and more likely to be normal weight (and underweight) compared to those with normal or HPV-negative ASCUS cytology (p=.003). There was no statistically significant difference in reported cigarette smoking (p=.111) between women with the outcome and those without the outcome.

No outcomes were observed among 101 visits among non-crack/cocaine-using, HIV-uninfected women. We observed five outcomes out of 157 visits (3.18%) among crack/cocaine-using HIV-uninfected women. Among HIV-infected women, we observed 13/142 outcomes (9.15%) among non-crack/cocaine-users and 31/225 outcomes (13.78%) among crack/cocaine users (p=0.18).

Table 3 shows the results of logistic regression analyses using GEE in this subset of 190 women. Overall, we observed an increased risk of incident HPV-positive Pap abnormality associated with crack/cocaine use in the unadjusted model, but this was of marginal statistical significance (OR=1.85, 95% C.I.: 0.93-3.66). Because there were no outcomes observed among non-crack/cocaine-exposed HIV-uninfected women, we were unable to estimate risk associated with crack/cocaine use in this subgroup. Among HIV-seropositive women, we observed a positive, but not statistically significant unadjusted association between recent crack/cocaine use and incident HPV-positive cytological abnormality (OR=1.58, 95% C.I.: 0.78-3.21). Adjustment for age, self-reported history of STIs, cigarette smoking, and BMI attenuated odd ratio estimates towards the null among the overall cohort and among HIV-infected women (OR=1.41, 95% C.I.:0.66-2.99), and this estimate remained consistent even with additional adjustment for ARV use.

Discussion

This study of former and current drug-using women in Baltimore found only marginal evidence for an association between recent cocaine use and incident cervical cytological abnormalities among HIV-infected and –uninfected women. We observed a non-statistically significant odds ratio of 1.78 among HIV-negative women; however, this estimate was diminished substantially after adjustment for multiple potential confounders. Among HIV-positive women in our study, there was no evidence of an association between recent cocaine use and incident Pap abnormalities.

Our results are in contrast to a previous study using a small subgroup of this cohort(3) where the investigators identified recent crack use to be associated with a statistically-significant, 1.7-fold increased odds of newly detected HPV infection among both HIV-positive and –negative women. In our study, drawing from the same cohort with more women and longer follow-up time, we observed risk estimates for incident HPV-positive cytological abnormalities among HIV-negative women in the same positive direction, but no increased risk was associated with cocaine exposure among HIV-positive women ($OR_{adjusted}$=1.01). Similarly to previous findings in this cohort(3), Minkoff et al reported a non-statistically significant, positive association between crack/cocaine use in the previous six months and risk of incident HPV-positive cervical lesions (OR=1.51, 95% C.I.: 0.99-2.30) among women with similar HIV-associated risk factors throughout the U.S.(4). However, the outcome in our study included minimally abnormal cytological changes in addition to the cervical lesions used as the outcome in the previous studies, and may reflect something other than HPV-associated cytological changes.

One possible explanation for our inability to replicate the prior findings reported by other investigators is that there is no true association between cocaine and incident cytological abnormalities and the prior findings were spurious. A second possibility that is supported by the results of our sensitivity analysis which indicated a stronger association similar to earlier results (3, 4), is that there may have been misclassification of the outcome in our analysis. It is possible that cytological abnormality detected by Pap smear (the primary outcome of interest) may not have reflected HPV–related cellular changes in all cases. Unfortunately, despite the suggestion of an association, we had limited power to detect a statistically significant association in HIV positive women and were unable to run multivariate analysis in HIV negative women. Taken together, these findings suggest that cocaine may be associated with HPV-associated clinical outcomes, but not cytological abnormalities without HPV.

The high prevalence of abnormal Pap results without concurrent HPV infection in our main analysis may be related to the older age of our study population; the ALIVE cohort is aging

with a median age of participants of 39 years at study entry. Results from the ATHENA study (14-16) and Kaiser study (17-19) support an age-related decrease in concordance of HPV positivity and Pap abnormality. In addition, an inverse association between the prevalence of a high-risk HPV infection among ASCUS Pap results and increasing age has been previously reported, where 74% of ASCUS results in women under 30 years were HPV positive and 19% in women 50 years and older, suggesting a distribution shift by age (20). An age effect on distribution of cytological abnormalities is also supported by biological mechanisms suggested to explain the increased incidence of cellular abnormalities with age, including immune senescence associated with aging and regression of the cervical transformation zone into the os in older women (17). Hormonal changes with increasing age may also elevate abnormal cytological findings and produce artifacts that resemble HPV effects (21, 22). Our data may provide further support to this hypothesis that cytological abnormalities detected by Pap smear in older women may represent morphological changes not associated with risk of HPV-associated cervical cancer.

This study has some limitations. First, we had limited power when restricting the outcome to HPV-associated cytological abnormalities. We were powered to detect an association of 1.6 for the 277 women in the overall analysis, but this power was attenuated in the sensitivity analysis with only 190 women, particularly among HIV negative women. While our results were suggestive of an association between crack/cocaine use and HPV positive Pap abnormalities, we cannot draw definitive conclusions because of small sample size and inability to perform multivariate analysis among HIV negative women. Secondly, measurement of Pap abnormality at semiannual visits (typically six months apart or longer) is an interval censored survival outcome (23), which could lead to missed HPV-associated transient cytological changes since a large proportion of ASCUS results are known to spontaneously regress in young, healthy women (24). As a result, our ability to detect any true association of crack/cocaine with clinically-detectable HPV-associated disease may have been limited, though the clinical relevance of these transient abnormalities is uncertain. Third, we acknowledge that exposures were self-reported by study

participants. While sexual and drug risk behaviors may be subject to reporting bias, we believe that the long-term nature of the parent study and rapport built between participants and study staff over time, as well as use of computerized methods to collect sensitive information in recent years, minimizes the possibility of this bias. Fourth, the population of current and former drug-using women included in this study is representative of chronic drug-using women in an urban community with high poverty rates and may not be representative of drug-using women in the overall United States. However, any biological mechanism underlying the purported association between cocaine use and cytological abnormalities should not be unique to urban-dwelling, drug-using women.

This study also has several strengths. To date, there are few studies focusing on crack/cocaine as a potential direct cause of HPV-associated clinical abnormalities. Using existing data, we highlighted the presence of and potential impact of non-HPV-associated abnormalities on analyses of Pap outcomes among older women. This study draws strength from the longitudinal design of a well-established parent study(7) and considerable increase in follow-up time, over 18 years, compared to previous analyses. Additionally, this community-based parent study gives a more accurate picture of the experience of women who do not necessarily have access to health care (but are referred to care as part of this study) and use cocaine.

In summary, we did not identify a clear association between recent cocaine use and incident cervical cytological abnormalities among a community-based cohort of HIV-infected and –uninfected drug-using women. This highlights the need for additional studies to utilize a better measure of HPV-associated cytological abnormality and an even larger population of HIV-uninfected cocaine-using women to elucidate any cocaine-associated effect on clinically-detectable HPV infection. Further, due to the known immune suppression associated with HIV infection and the high prevalence and incidence of abnormal cytology among HIV-infected women(25, 26), future work among drug-using populations with and at-risk for HIV should

stratify by HIV status to remove the strong effect of HIV on analyses of immune-system-related outcomes, specifically when examining HPV and abnormal cytology.

References

1. Substance Abuse and Mental Health Services Administration. Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2013 Contract No.: HHS Publication No. (SMA) 13-4795.
2. Kay A, Taylor TE, Barthwell AG, Wichelecki J, Leopold V. Substance use and women's health. J Addict Dis. 2010;29(2):139-63.
3. Phelan DF, Gange SJ, Ahdieh-Grant L, Mehta SH, Kirk GD, Shah K, et al. Determinants of Newly Detected Human Papillomavirus Infection in HIV-Infected and HIV-Uninfected Injection Drug Using Women. Sex Transm Dis. 2009.
4. Minkoff H, Zhong Y, Strickler HD, Watts DH, Palefsky JM, Levine AM, et al. The relationship between cocaine use and human papillomavirus infections in HIV-seropositive and HIV-seronegative women. Infect Dis Obstet Gynecol. 2008;2008:587082.
5. Serraino D, Napoli PA, Zaccarelli M, Alliegro MB, Pezzotti P, Rezza G. High frequency of invasive cervical cancer among female injecting drug users with AIDS in Italy. Aids. 1996;10(9):1041-2.
6. Dinh TH, Sternberg M, Dunne EF, Markowitz LE. Genital warts among 18- to 59-year-olds in the United States, national health and nutrition examination survey, 1999--2004. Sex Transm Dis. 2008;35(4):357-60.
7. Vlahov D, Anthony JC, Munoz A, Margolick J, Nelson KE, Celentano DD, et al. The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. NIDA Res Monogr. 1991;109:75-100.
8. The Bethesda System for reporting cervical/vaginal cytologic diagnoses: revised after the second National Cancer Institute Workshop, April 29-30, 1991. Acta Cytol. 1993;37(2):115-24.
9. Pan W. Akaike's information criterion in generalized estimating equations. Biometrics. 2001;57(1):120-5.
10. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics. 1986;42(1):121-30.
11. Shah KV, Solomon L, Daniel R, Cohn S, Vlahov D. Comparison of PCR and hybrid capture methods for detection of human papillomavirus in injection drug-using women at high risk of human immunodeficiency virus infection. J Clin Microbiol. 1997;35(2):517-9.
12. Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst. 2005;97(8):577-86.
13. Castle PE, Cox JT, Jeronimo J, Solomon D, Wheeler CM, Gravitt PE, et al. An analysis of high-risk human papillomavirus DNA-negative cervical precancers in the ASCUS-LSIL Triage Study (ALTS). Obstet Gynecol. 2008;111(4):847-56.
14. Wright TC, Jr., Stoler MH, Behrens CM, Apple R, Derion T, Wright TL. The ATHENA human papillomavirus study: design, methods, and baseline results. Am J Obstet Gynecol. 2012;206(1):46 e1- e11.
15. Stoler MH, Wright TC, Jr., Sharma A, Apple R, Gutekunst K, Wright TL. High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. Am J Clin Pathol. 2011;135(3):468-75.
16. Stoler MH, Wright TC, Jr., Sharma A, Zhang G, Apple R, Wright TL, et al. The interplay of age stratification and HPV testing on the predictive value of ASC-US cytology. Results from the ATHENA HPV study. Am J Clin Pathol. 2012;137(2):295-303.
17. Castle PE, Jeronimo J, Schiffman M, Herrero R, Rodriguez AC, Bratti MC, et al. Age-related changes of the cervix influence human papillomavirus type distribution. Cancer Res. 2006;66(2):1218-24.

18. Littell RD, Kinney W, Fetterman B, Cox JT, Shaber R, Poitras N, et al. Risk of cervical precancer and cancer in women aged 30 years and older with an HPV-negative low-grade squamous intraepithelial lesion screening result. J Low Genit Tract Dis. 2011;15(1):54-9.
19. Castle PE, Fetterman B, Poitras N, Lorey T, Shaber R, Kinney W. Five-year experience of human papillomavirus DNA and Papanicolaou test cotesting. Obstet Gynecol. 2009;113(3):595-600.
20. Silverloo I, Andrae B, Wilander E. Value of high-risk HPV-DNA testing in the triage of ASCUS. Acta Obstet Gynecol Scand. 2009;88(9):1006-10.
21. Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA. 2001;285(11):1500-5.
22. Morrison C, Prokorym P, Piquero C, Wakely PE, Jr., Nuovo GJ. Oral contraceptive pills are associated with artifacts in ThinPrep Pap smears that mimic low-grade squamous intraepithelial lesions. Cancer. 2003;99(2):75-82.
23. Kong X, Gray RH, Moulton LH, Wawer M, Wang MC. A modeling framework for the analysis of HPV incidence and persistence: a semi-parametric approach for clustered binary longitudinal data analysis. Stat Med. 2010;29(28):2880-9.
24. ACOG Practice Bulletin no. 109: Cervical cytology screening. Obstet Gynecol. 2009;114(6):1409-20.
25. Massad LS, Riester KA, Anastos KM, Fruchter RG, Palefsky JM, Burk RD, et al. Prevalence and predictors of squamous cell abnormalities in Papanicolaou smears from women infected with HIV-1. Women's Interagency HIV Study Group. J Acquir Immune Defic Syndr. 1999;21(1):33-41.
26. Massad LS, Ahdieh L, Benning L, Minkoff H, Greenblatt RM, Watts H, et al. Evolution of cervical abnormalities among women with HIV-1: evidence from surveillance cytology in the women's interagency HIV study. J Acquir Immune Defic Syndr. 2001;27(5):432-42.

Table 1. Index Visit Characteristics of 277 Female Participants by Recent Cocaine or Crack Cocaine Use, The AIDS Linked to the Intravenous Experience (ALIVE) study

	No cocaine or crack use		Any cocaine or crack use		Total	p-value*
Characteristic	N	%	N	%	N	
Total	114	41.0	163	59.0	277	
Demographics						
Age category (years)						
<35	28	24.6	46	28.2	74	0.20
35-39	33	29.0	55	33.7	88	
40-45	21	18.4	34	20.9	55	
>45	32	28.1	28	17.2	60	
Race						
White	9	7.9	4	2.5	13	0.13
Black, non-Hispanic	101	88.6	153	93.9	254	
Black, Hispanic	2	1.8	5	3.1	7	
Asian	2	1.8	1	0.6	3	
Cohort (year enrolled)						
10,000s (1988-1989)	77	67.5	127	77.9	204	0.18
70,000s (1994-1995)	20	17.5	15	9.2	35	
80,000s (1998)	8	7.0	6	3.7	14	
20,000s (2000-2001)	6	5.3	10	6.1	16	
30,000s (2005)	3	2.6	5	3.1	8	
Clinical/Immunologic Characteristics						
Body Mass Index, kg/m^2						
Underweight, <18.5	4	3.5	14	8.6	18	<.0001
Normal weight, 18.5-24.9	39	34.5	101	62.4	140	
Overweight, 25-29.9	38	33.6	28	17.3	66	
Obese, ≥30	32	28.3	19	11.7	51	
HIV serostatus / CD4 category						
Uninfected	39	34.2	50	30.7	89	0.24
HIV+, CD4 >500	32	28.1	34	20.9	66	
HIV+, CD4 200-500	29	25.4	47	28.8	76	
HIV+, CD4 < 500	14	12.3	32	19.6	46	

Characteristic		No cocaine or crack use N	%	Any cocaine or crack use N	%	Total N	p-value*
Prior antiretroviral therapy							
	No	44	59.5	68	60.2	112	0.92
	Yes	30	40.5	45	39.8	75	
Prior AIDS diagnosis							
	No	85	74.6	105	64.4	190	0.07
	Yes	29	25.4	58	35.6	87	
Behavioral Risk Factors							
Cigarette smoking							
	No	15	13.2	11	6.7	26	0.07
	Yes	99	86.8	152	93.3	251	
Any sexual intercourse							
	No	39	34.2	59	36.2	98	0.77
	Yes	74	64.9	104	63.8	178	
Sexual intercourse with IDU							
	No	84	77.8	93	59.6	177	0.002
	Yes	24	22.2	63	40.4	87	
Sexual intercourse with anonymous partner							
	No	107	99.1	143	91.7	250	0.0096
	Yes	1	0.9	13	8.3	14	
Number of male sexual partners							
	0-2	110	99.1	144	88.9	254	0.0017
	3-7	1	0.9	15	9.3	16	
	>7	0	0	3	1.9	3	
Sexually Transmitted Infections‡							
	No	104	91.2	135	83.3	239	0.06
	Yes	10	8.8	27	16.7	37	

Characteristic	No cocaine or crack use N	%	Any cocaine or crack use N	%	Total N	p-value*
Substance Use Behavior						
Injection drug use						
No	111	83.5	22	8.5	133	<.0001
Less than daily	15	11.3	96	37.2	111	
At least daily	6	4.5	138	53.5	144	
Marijuana use						
No	98	86.0	106	65.0	204	<.0001
Yes	16	14.0	57	35.0	73	
Heroin use						
No	97	85.1	15	9.2	112	<.0001
Yes	17	14.8	148	90.8	165	
Shared needles						
No	113	99.1	114	70.9	227	<.0001
Yes	1	0.9	49	30.1	50	

Notes: Characteristics refer to the six-month time period prior to baseline visit, unless otherwise mentioned. ASCUS=Atypical Squamous Cells of Undetermined Significance; LSIL=Low-grade Squamous Intraepithelial Lesions; HSIL=High-grade Squamous Intraepithelial Lesions; IDU=Injection Drug Use.
Percentages may not sum to 100% due to missing observations and/or rounding.

* p-values are based on chi-square test for categorical variables or Fisher's exact test for variables with cell values less than five.

† HPV type ascertained on a subset of women who provided cervicovaginal lavage specimens for a separate substudy (227 missing values at baseline visit).

‡ STIs reported do not include Chlamydia.

Table 1a. Cervical Cytology and HPV Type Status of Female Participants by Recent Cocaine or Crack Cocaine Use at Index Visit, The AIDS Linked to the Intravenous Experience (ALIVE) study

Characteristic	No cocaine or crack use N	(%)	Any cocaine or crack use N	(%)	Total	p-value*
Total	114	41.0	163	59.0	277	
Incident Pap abnormality						
No	101	88.6	138	84.7	239	0.35
Yes	13	11.4	25	15.3	38	
Pap smear result						
Normal	101	88.6	138	84.7	239	0.39
ASCUS	7	6.1	19	11.7	26	
LSIL	3	2.6	4	2.5	7	
HSIL	3	2.6	2	1.2	5	
HPV type detected†						
Oncogenic	8	44.4	14	43.8	22	0.96
Non-oncogenic	10	55.6	18	56.3	28	

Notes: Characteristics refer to the six-month time period prior to baseline visit, unless otherwise mentioned. ASCUS=Atypical Squamous Cells of Undetermined Significance; LSIL=Low-grade Squamous Intraepithelial Lesions; HSIL=High-grade Squamous Intraepithelial Lesions; IDU=Injection Drug Use.

Percentages may not sum to 100% due to missing observations and/or rounding.

* p-values are based on chi-square test for categorical variables or Fisher's exact test for variables with cell values less than five.

† HPV type ascertained on a subset of women who provided cervicovaginal lavage specimens for a separate substudy (227 missing values at baseline visit).

Table 2. Odds ratios (OR) and 95% confidence intervals of incident cervical cytological abnormality for any recent crack/cocaine use from logistic regression with generalized estimating equations (GEE) in 277 women, The AIDS Linked to the Intravenous Experience (ALIVE) study

Model Specification	Visits	Women	No Crack/Cocaine OR	No Crack/Cocaine 95% C.I.	Any Crack/Cocaine OR	Any Crack/Cocaine 95% C.I.
Unadjusted Model						
Overall cohort	1864	277	Ref		1.23	0.93-1.63
Among HIV-Negative	810	89	Ref		1.78	0.95-3.34
Among HIV-Positive	1054	198	Ref		1.09	0.77-1.54
Adjusted Model (adjusting for age, self-reported history of sexually transmitted infections, cigarette smoking, body mass index)						
Overall cohort	1844	276	Ref		1.06	0.79-1.44
Among HIV-Negative	800	89	Ref		1.29	0.65-2.57
Among HIV-Positive	1044	197	Ref		1.01	0.69-1.48

Table 3. Odds ratios (OR) and 95% confidence intervals of incident HPV-positive cytological abnormality for any recent crack/cocaine use from logistic regression with generalized estimating equations (GEE) in subset of 190 women with HPV genotype testing, The AIDS Linked to the Intravenous Experience (ALIVE) study

Model Specification			No Crack/Cocaine		Any Crack/Cocaine	
	Visits	Women	OR	95% C.I.	OR	95% C.I.
Unadjusted Model						
Overall cohort	625	190	Ref		1.85	0.93-3.66
Among HIV-Negative				Non-estimable*		
Among HIV-Positive	367	126	Ref		1.58	0.78-3.21
Adjusted Model (adjusting for age, self-reported history of sexually transmitted infections, cigarette smoking, body mass index)						
Overall cohort	620	190	Ref		1.42	0.71-2.85
Among HIV-Negative				Non-estimable*		
Among HIV-Positive	365	126	Ref		1.41	0.66-2.99

*There were zero outcomes observed among 101 visits among non-crack/cocaine-using, HIV-seronegative women. We observed five outcomes out of 157 visits (3.18%) among crack/cocaine-using HIV-seronegative women. In contrast, among HIV-seropositive women, we observed 13/142 outcomes (9.15%) among non-crack/cocaine-users and 31/225 outcomes (13.78%) among crack/cocaine users.

Chapter Four

Discussion

Synthesis of evidence

Current Evidence

Current understanding of the natural history of human papillomavirus (HPV) infection involves the virus' ability to evade the host immune system and replicate, typically resulting in a transient infection when immunity is intact (1). The role of host immunity is critical in controlling HPV replication and preventing development of detectable cervical precancerous lesions (2, 3). However, other risk factors have been identified as being associated with increased risk of HPV infection and related outcomes including cervical lesions and cervical cancer; these include sexual risk behavior, illicit drug use, and dampened immune response (2). In addition, there is growing evidence that drug use, especially use of crack/cocaine, may be associated with increased risk of HPV and cervical cancer through an immunologic mechanism (4-6) independently from shared sexual risk. Prior epidemiologic research has not focused on characterizing the potential risk associated with cocaine use as an immune-dampening exposure and the potential impact of cocaine use on HPV infection; the work described in this dissertation examines the hypothesis that cocaine use is associated with HPV-associated clinical outcomes through an immunologic mechanism.

Cocaine use and Clinically Detectable HPV Infection

To examine the potential association between cocaine use and HPV infection, we focused on clinically detectable infections, observable as abnormal Pap results of atypical squamous cells of undetermined significance (ASCUS) or more severe cytological abnormality among HIV-infected and uninfected women in an urban community-based cohort followed over 18 years. We observed a positive association between recent

cocaine use by any route of administration and risk of incident cervical cytological abnormality in the overall cohort. The association is likely driven by the risk among HIV-infected women, which comprise a majority of the analytic cohort (70%), since there are no observable outcomes in HIV-infected women who have intact immune systems (Chapter 3). While prior research has established a positive association between drug use and HPV infection among cohorts of HIV infected and uninfected women (7, 8), our findings did not achieve statistical significance. With zero HPV clinical outcomes detected among uninfected women, we were unable to examine this potential association in a setting independent from the immunosuppressed state caused by HIV infection. Despite this limitation, some comments can be made. The lack of events observed among uninfected women in this population was likely due to the reduced sample size and low risk of HPV-clinical outcomes in this immune competent population, which might be interpreted as the expected occurrence of abnormality among populations with an intact adaptive immunity and no exposure to immune-modulating cocaine. However, we did observe a moderate association in the hypothesized direction of increased risk among drug-using HIV-infected women (OR=1.41, 95% C.I.: 0.66-2.99), which lends support for the possibility that cocaine use is truly associated with increased risk of HPV-related outcomes.

Cocaine-induced Immune Suppression

We proposed that the known association between cocaine and HPV infection may occur through a hypothesized mechanism by which cocaine elicits an immunosuppressive response in drug users. Since an HPV-specific adaptive immune response is critical to lesion regression (9), it is possible that cocaine use causes immune dampening which

may result in uncontrolled HPV replication. Additional evidence supporting this mechanism is provided in a different cohort of at-risk HIV-positive and HIV-negative women throughout the United States, the Women's Interagency HIV Study (WIHS). In a subset of the overall WIHS cohort followed prior to the broad usage of highly active antiretroviral therapy (HAART), we observed statistically significant evidence of reduced adaptive immune response at the epithelial cell level among HIV-uninfected cocaine users (Chapter 2). Since HPV is an exclusively intraepithelial infection (9), our findings provide additional support for plausibility of a biological mechanism where cocaine-induced immune suppression may increase the risk of HPV-associated clinical outcomes.

Differential Anergy Results Among HIV-Infected and Uninfected Women

Interestingly, we observed a positive association between cocaine use and local epithelial immune response only among HIV-uninfected women in the WIHS. Among women with HIV infection, cocaine use was not associated with anergy in either unadjusted (OR_{unadj} 1.03, 95% C.I.: 0.80-1.34) or adjusted analyses (OR_{adj} 1.08, 95% C.I.: 0.78-1.50). It is plausible that any cocaine-induced immune dampening may be masked by overwhelming HIV-associated systemic immune suppression, resulting in the contrasting results we observed between HIV-infected and uninfected populations. In our analytic cohort, the 277 HIV-infected women displayed characteristically low systemic immunity indicated by a median CD4 cell count of 391 (IQR: 207-612), while uninfected women had higher cell counts (median 1,007, IQR: 818-1,341) reflecting intact immunity. Examining associations among HIV-infected and uninfected populations separately likely enabled this association to be elucidated among uninfected women, if this mechanism is indeed true. In unadjusted analysis of the cocaine-anergy relationship

among WIHS women, those with very low CD4 cell counts (less than 200 cells/mm^3) were more likely to be anergic compared to those with higher CD4 counts (Chapter 2, Table 2). Results from the bivariate analyses among HIV-infected women, where anergy was found to be associated with overall systemic immune suppression, provide additional evidence that cocaine's effect on anergy may be washed out by HIV-associated immune suppression. In future work, stratification by CD4 cell count would be pertinent to examine systemic immune influences on the cocaine-anergy relationship, but unfortunately, we were underpowered to do so in these analyses.

Cocaine and Other Illicit Drug Use

An additional challenge in attempting to isolate the effect of one drug exposure use is that cocaine is commonly used in combination with other illicit drugs. In the ALIVE cohort, we found that 65% of the women who reported cocaine use injected a combination of cocaine and heroin (also known as "speedball" injection), highlighting the difficulty of disentangling the effects of cocaine from heroin and other drugs. In the WIHS, we also observed a high level of combined drug use: 40% of recent cocaine users also reported using heroin within the last six months and half of cocaine users also used marijuana. In terms of psychoactive effects, cocaine is a stimulant while heroin is a depressant, yet each dampen immune responses. Opiates like heroin have been suggested to have indirect immunomodulatory effects through interactions with the neuroendocrine system(10, 11) as opposed to the direct effect of cocaine on cytokines and immune cells(5, 12, 13). Teasing apart the effect of a single drug type presents a challenge since exposure to either drug results in immune response suppression in animal (14-16) and

cell line models (17). Given the similar immune effects, we would expect to observe additive risk of immune reductions among combination drug users.

Applying this logic to our ALIVE analyses, we would have expected that any combination drug use in addition to cocaine would be likely to inflate the overall observed association, since increases in the risk of immune suppression would be expected with multiple immune-suppressive drug exposures, subsequently allowing for increased HPV persistence. However, when we examined the effect of exposure to both cocaine and heroin on risk of any incident cervical cytological abnormality compared to use of cocaine only, we did not observe any notable differences in effect size between the resulting risk estimates in the ALIVE cohort (data not shown). Benefitting from available data on recent use of a variety of illicit drugs, our research was able to account for other types of drugs used including marijuana and heroin. In this dissertation, we focus on a single drug type; however, we emphasize that use of any immune-suppressive drug may be a contributing risk factor for HPV persistence and associated disease.

Role of sexual risk behavior on observed associations

Existing published literature suggests that shared sexual risk behavior accounts for the increased risk of HPV-related disease among drug-using populations by increasing exposure to HPV infection (8, 18). However, there is evidence that sexual risk alone may not explain the entire association; in the control arm of a quadrivalent HPV vaccine efficacy study, incident HPV detection was observed among recently non-sexually active participants (19). Additionally, in a previous, smaller study among HIV-uninfected women in one of our cohorts (ALIVE), accounting for sexual risk behavior did not attenuate risk estimates for crack/cocaine use and HPV-related outcomes (7). In

our expanded study on ALIVE women followed for nearly two decades, indicators of recent sexual risk including number of male sexual partners and high-risk sexual intercourse similarly did not have a notable impact on the observed association between cocaine use and incident HPV-positive abnormal Pap results. As a result, the only sexual risk factor retained in the multivariate adjusted model was self-reported history of sexually transmitted infections (STIs), which may be a marker of previous exposure to HPV rather than purely reflective of recent sexual risk behavior. Furthermore, adjustment for history of STIs along with other potential confounders resulted only in a slight attenuation of the risk estimate among HIV-infected women, OR=1.58 vs. 1.41, with the same positive direction of association retained. In our research, we did not find evidence for confounding by sexual risk, nor did sexual risk factors play a substantial role in dampening the magnitude of observed effect size. Therefore, we cannot conclude that sexual risk behavior explains much of the increased risk of HPV-associated abnormalities, and, fittingly, our findings do not rule out an alternate mechanism of cocaine-induced genital tract immune suppression.

Conclusion

In this research using data from ALIVE and WIHS women, we hypothesized that local epithelial immune suppression directly related to cocaine exposure may allow for HPV infection to proliferate and manifest as clinically detectable cellular changes in cervical epithelial tissue. Even though our findings were not definitive, this work is persuasive in calling attention to the potential contribution of cocaine use to local immune suppression and the subsequent impact on HPV natural history. While we were unable to show conclusive evidence for this purported association among HIV-infected

women due to power constraints, our findings do suggest that an immune suppressive effect of cocaine use may be real. Besides our study, the association between cocaine use and HPV infection has only been previously examined in two studies which have identified associations between recent crack/cocaine use and new HPV detection (OR, 1.7; 95% CI, 1.1-2.6) (7), prevalent detection of oncogenic HPV (OR, 1.30; 95% C.I.: 1.09-1.55), prevalent detection of oncogenic HPV-positive SIL (OR, 1.70; 95% C.I.: 1.27-2.27), and a non-statistically significant risk of incident oncogenic HPV-positive SIL (HR, 1.51; 95% CI 0.99-2.30) (8). Our findings, in conjunction with these previous studies, provide growing epidemiologic evidence for a biological mechanism of cocaine-induced immune compromise which has the potential to increase risk of HPV persistence and, more rarely, progression to cervical cancer.

Limitations

We recognize that our results may not be generalizable to all women who use cocaine or crack cocaine, since cocaine use may not have a clinically relevant effect among severely immune compromised HIV-infected women. Despite this, we believe it is plausible that a biological effect of cocaine-associated suppression of immune response to HPV infection would be similar among other immune competent drug-using populations. Since recruitment methods from ALIVE enrolled participants directly from the community, inferences drawn from data from the ALIVE study on cocaine use and abnormal Pap outcomes may be generalizable to similar injection drug users (IDUs) in Baltimore; however, since IDU practices have been reported to differ by urban location(20), we may not be able to generalize our findings to other drug-users in different areas. Nevertheless, since we believe that a biological mechanism of immune

suppression underlies the association observed between cocaine use and HPV-associated lesions, we would not expect this mechanism to differ across geographic areas and we believe our findings may be generalizable to cocaine-exposed women. In addition, the data available on anergy test results were collected between 1994 and 1996; while this is data is not current, the biological mechanism we are interested in has likely not changed over time.

Our findings may also be limited by the timing of measurement of the HPV-associated clinical outcomes since a large proportion of ASCUS results are known to spontaneously regress in young, healthy women(21). In the ALIVE, measurement of Pap outcomes at semiannual visits occur typically at six month intervals, or even longer as visits are often missed among drug using populations. Measuring this interval-censored survival outcome(22) could theoretically lead to missed HPV-associated transient cytological changes. It is possible that, as described, non-differential misclassification of outcome may have limited our ability to detect a true association of crack/cocaine and increased risk of HPV disease. Similar to other cohort studies employing semi-annual visits, this study design did not allow for measurement of outcomes at shorter time intervals due to logistical constraints and undue burden on participants. For this analysis, we focused on clinically detectable cervical cytological changes to reflect increased risk of progression to cervical cancer. Including minimally abnormal Pap results (e.g., ASCUS) in the outcome definition may have inappropriately captured typically transient changes that may not correlate with cervical cancer risk. Nevertheless, we believe that measuring Pap results at extended intervals ensures that positive outcomes reflect HPV infection that has persisted long enough to cause clinically detectable changes. Thus, our

results should be relevant when interpreted as the cocaine-associated risk of HPV disease progression.

Throughout these analyses, measures of exposure and various covariates were assessed through participant self-report. We have no data on biological measures to confirm these self-reported data on drug use; however, behaviors are reported using audio computer-assisted self-interviewing (ACASI) in the ALIVE cohort, which has been shown to improve disclosure of illicit drug use and HIV-related risk behaviors (23, 24). In addition, self-reported drug use practices have been validated in other similar natural history studies, as well as a review (24-26). If drug users were systematically underreporting use of cocaine due to social desirability bias, then we would expect that any associations observed would be biased towards the null. Therefore, our findings would reflect a conservative estimate of the association between cocaine use and HPV-related clinical outcomes.

Strengths

Our research using data from two different cohorts provides initial evidence in support of drug-induced immune suppression as a mechanism for loss of immune control of HPV infection. The interdisciplinary nature of linkages between illicit drug use behavior, basic science mechanisms, HPV viral persistence and replication, cervical and mucosal immunology, and clinical measures of our outcomes were drawn together in this research to better understand the complex nature of HPV natural history and cocaine use. We benefit from having high quality data from which to draw inferences by studying these associations in two prospective cohorts followed for many years. These longitudinal studies have successfully recruited and retained populations who are difficult to identify,

and are robust sources of sensitive information on drug use and sexual risk behavior that are challenging to collect. Additionally, our studies focused on populations that are poised to benefit from focused research attention; the ALIVE population is predominantly African-American and represents an understudied group, while both WIHS and ALIVE provide data that reflect the real-world experience of drug-using women(20, 27) who represent a vulnerable population.

Our findings are novel because we provide initial epidemiologic support to build a case for the hypothesis that clinically detectable HPV outcomes may be partially explained by drug-induced anergy. We utilized existing cohort data to directly assess the association between cocaine use and cutaneous anergy, and explore whether cocaine use is associated with increases in clinical evidence of HPV replication (i.e., SIL). By focusing on clinically relevant outcomes, we hope to ensure that our results are applicable to clinical management of these vulnerable populations.

Public health significance

In the United States, an estimated 14.9 million females report any cocaine use in their lifetime and 1.37 million women used cocaine in the past year based on 2012 data (28). Despite the large size of this drug-using population, the impact of cocaine use on HPV natural history has not been well studied. Since cervical cancer is preventable, there is substantial benefit that can be achieved with identification of populations at higher risk for HPV-associated disease, specifically those at risk due to immune suppressive effects of cocaine use. Current cervical cancer guidelines do not specify differential screening practices based on drug use, but have recently suggested more frequent screening among HIV-infected and immune compromised populations (29). Given our findings, a more

thorough understanding of cocaine-associated HPV risk could potentially support clinicians and public health professionals to continue work towards achieving adequate cervical pre-cancer surveillance among cocaine-using populations and inform appropriate clinical management of HPV infection among women who use drugs.

Improved understanding of the role of HPV infection in cervical cancer risk and the potential impact of cocaine use on HPV disease risk among drug users may lead to a desire to mitigate risk of cervical cancer by participating in enhanced surveillance or reducing drug use. Community outreach opportunities including health education among drug users to increase knowledge of HPV risk (30) may be implemented to encourage risk reduction among cocaine-using women. In addition, these findings may guide public health programs to consider focusing efforts on supporting drug cessation in addition to health education to reduce risk of HPV-associated disease.

The results from our studies indicate that cocaine-associated immune suppression may play an important role in HPV natural history in these exceptionally vulnerable populations. Enhanced understanding of the nature of and risk related to replicative HPV infection may also help public health professionals to target and plan interventions for reducing HPV transmission by drug-using core groups with active HPV infection.

Future work

Current gaps in knowledge surrounding HPV natural history include the need to identify markers of reduced immunity and correlates of HPV-associated disease outcomes (31). In our work, we used cutaneous anergy as a proxy measure of epithelial immune response at the cervix. It would better address HPV-specific research questions if future studies had a specific marker or measure of mucosal epithelial immunity at the

cervical endothelium, the preferred target for HPV infection(32), to minimize potential for bias.

Our work highlights opportunities where future research may help clinicians and public health professionals caring for women who use drugs by improving risk stratification for those at risk for HPV-associated outcomes. A more thorough understanding of the complex relationship between cocaine use, immune-suppression, and HPV disease could be gained through larger prospective studies of HIV-uninfected populations who use cocaine. We would also benefit from studies in which measures of cocaine exposure, HPV-specific immune markers, and HPV outcomes are taken within in the same cohort in order to examine the role of immune suppression as a mediator of the association between cocaine use and HPV-related disease.

To gain a better understanding of the cocaine-mediated pathway of local immune suppression, future work should explore additional specifications of exposure, including intermittent cocaine use compared to long-term or other patterns of drug use. Such studies may elucidate if varying patterns of use differentially affect immune responses, specifically control of HPV infection and subsequent clinical sequelae. However, we acknowledge that such studies but would require large populations with detailed data on drug use dosage, frequency, and usage patterns followed for a long period of time in order to be sufficiently powered to examine these additional refinements to the exposure-outcome relationship.

We learned from our analyses that interpreting cervical abnormalities detected on Pap smear in an aging population of women is challenging. Abnormal Pap results may not always reflect HPV infection and may simply signify age-related changes(33, 34).

With this in mind, understanding cocaine-associated risk for HPV-associated disease could also benefit from research using HPV DNA testing for outcome measurement, which is a more sensitive test than the Pap smear for detecting cervical intraepithelial neoplasia (35, 36). In addition, HPV genotype may be better correlated with cervical cancer risk than cytology (37), as high-risk HPV types have been associated with increased likelihood of persistence and progression to cervical cancer (38). Thus, using HPV genotype to define outcome may be better for making inferences about cervical cancer risk, which is the ultimate goal for prevention. Additionally, data on specific HPV genotype may enable further stratification of cocaine users by high- and low-risk HPV groups, resulting in tailored screening programs and adapted clinical management among drug users.

Studies that collect and validate HPV genotypes detected over time could also contribute to distinguishing between existing and new infection. By doing so, this may improve understanding of the potential impact of cocaine on immune control of latent HPV infection, which may be identified as an existing genotype that becomes undetectable and is subsequently re-detected. It is possible that additional findings supporting cocaine-associated immune effects on HPV would generate supporting evidence for the hypothesized latent state of HPV infection (39), which could then lead to changes in the current understanding of HPV natural history. However, such studies would be difficult to conduct as frequent follow-up with intervals that allow adequate capture of potentially transient outcome measures would require vast resources and high adherence, which may be difficult to achieve in drug-using populations.

Results from our research using data from the ALIVE and WIHS cohorts of HIV-infected and -uninfected women contribute evidence to support the possibility that cocaine use may induce immunosuppressive effects that impact persistence of HPV infection among otherwise immune competent women. Future research targeting this knowledge gap will help to better understand the cocaine-induced mechanism of immune-mediated alterations in HPV natural history. These proposed studies will be critical for improving strategies for risk stratification and prevention of cervical cancer among drug-using populations. More importantly, improved understanding of drug-induced local immune suppression will contribute to a larger scientific body of knowledge regarding the natural history of HPV infection and HPV-related cervical pathogenesis.

References

1. Moscicki AB, Schiffman M, Burchell A, Albero G, Giuliano AR, Goodman MT, et al. Updating the natural history of human papillomavirus and anogenital cancers. Vaccine. 2012;30 Suppl 5:F24-33.
2. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006;24 Suppl 1:S1-15.
3. Stanley M. Immune responses to human papillomavirus. Vaccine. 2006;24 Suppl 1:S16-22.
4. Friedman H, Pross S, Klein TW. Addictive drugs and their relationship with infectious diseases. FEMS Immunol Med Microbiol. 2006;47(3):330-42.
5. Lopez MC, Watson RR. Effect of cocaine and murine AIDS on lamina propria T and B cells in normal mice. Life Sci. 1994;54(9):PL147-51.
6. Watzl B, Watson RR. Immunomodulation by cocaine--a neuroendocrine mediated response. Life Sci. 1990;46(19):1319-29.
7. Phelan DF, Gange SJ, Ahdieh-Grant L, Mehta SH, Kirk GD, Shah K, et al. Determinants of Newly Detected Human Papillomavirus Infection in HIV-Infected and HIV-Uninfected Injection Drug Using Women. Sex Transm Dis. 2009.
8. Minkoff H, Zhong Y, Strickler HD, Watts DH, Palefsky JM, Levine AM, et al. The relationship between cocaine use and human papillomavirus infections in HIV-seropositive and HIV-seronegative women. Infect Dis Obstet Gynecol. 2008;2008:587082.
9. Stanley M. HPV - immune response to infection and vaccination. Infect Agent Cancer. 2010;5:19.
10. Alonzo NC, Bayer BM. Opioids, immunology, and host defenses of intravenous drug abusers. Infect Dis Clin North Am. 2002;16(3):553-69.
11. Kreek MJ. Immune function in heroin addicts and former heroin addicts in treatment: pre- and post-AIDS epidemic. NIDA Res Monogr. 1990;96:192-219.
12. Irwin MR, Olmos L, Wang M, Valladares EM, Motivala SJ, Fong T, et al. Cocaine dependence and acute cocaine induce decreases of monocyte proinflammatory cytokine expression across the diurnal period: autonomic mechanisms. J Pharmacol Exp Ther. 2007;320(2):507-15.
13. Ruiz P, Cleary T, Nassiri M, Steele B. Human T lymphocyte subpopulation and NK cell alterations in persons exposed to cocaine. Clin Immunol Immunopathol. 1994;70(3):245-50.
14. Roy S, Loh HH. Effects of opioids on the immune system. Neurochemical research. 1996;21(11):1375-86.
15. Risdahl JM, Khanna KV, Peterson PK, Molitor TW. Opiates and infection. J Neuroimmunol. 1998;83(1-2):4-18.
16. Weber RJ, Gomez-Flores R, Smith JE, Martin TJ. Immune, neuroendocrine, and somatic alterations in animal models of human heroin abuse. J Neuroimmunol. 2004;147(1-2):134-7.
17. Peterson PK, Sharp BM, Gekker G, Portoghese PS, Sannerud K, Balfour HH, Jr. Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures. AIDS. 1990;4(9):869-73.

18. Vlahov D, Safaien M, Lai S, Strathdee SA, Johnson L, Sterling T, et al. Sexual and drug risk-related behaviours after initiating highly active antiretroviral therapy among injection drug users. Aids. 2001;15(17):2311-6.
19. Velicer C, Zhu X, Vuocolo S, Liaw KL, Saah A. Prevalence and incidence of HPV genital infection in women. Sex Transm Dis. 2009;36(11):696-703.
20. Vlahov D, Anthony JC, Munoz A, Margolick J, Nelson KE, Celentano DD, et al. The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. NIDA Res Monogr. 1991;109:75-100.
21. ACOG Practice Bulletin no. 109: Cervical cytology screening. Obstet Gynecol. 2009;114(6):1409-20.
22. Kong X, Gray RH, Moulton LH, Wawer M, Wang MC. A modeling framework for the analysis of HPV incidence and persistence: a semi-parametric approach for clustered binary longitudinal data analysis. Stat Med. 2010;29(28):2880-9.
23. Macalino GE, Celentano DD, Latkin C, Strathdee SA, Vlahov D. Risk behaviors by audio computer-assisted self-interviews among HIV-seropositive and HIV-seronegative injection drug users. AIDS Educ Prev. 2002;14(5):367-78.
24. Turner CF, Villarroel MA, Rogers SM, Eggleston E, Ganapathi L, Roman AM, et al. Reducing bias in telephone survey estimates of the prevalence of drug use: a randomized trial of telephone audio-CASI. Addiction. 2005;100(10):1432-44.
25. Darke S. Self-report among injecting drug users: a review. Drug Alcohol Depend. 1998;51(3):253-63; discussion 67-8.
26. Landay A, Benning L, Bremer J, Weiser B, Burger H, Nowicki M, et al. Correlates of immune activation marker changes in human immunodeficiency virus (HIV)-seropositive and high-risk HIV-seronegative women who use illicit drugs. J Infect Dis. 2003;188(2):209-18.
27. Bacon MC, von Wyl V, Alden C, Sharp G, Robison E, Hessol N, et al. The Women's Interagency HIV Study: an observational cohort brings clinical sciences to the bench. Clin Diagn Lab Immunol. 2005;12(9):1013-9.
28. Administration SAaMHS. Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2013 Contract No.: HHS Publication No. (SMA) 13-4795.
29. Committee on Practice B-G. ACOG Practice Bulletin Number 131: Screening for cervical cancer. Obstet Gynecol. 2012;120(5):1222-38.
30. Bonney L, Cardenas G, Del Rio C, Hooton T, Metsch L, Rosa-Cunha I, editors. HPV knowledge among HIV-infected drug users in two urban areas. Infectious Disease Society of America; 2009 October 30, 2009; Philadelphia, PA.
31. Wheeler CM. The natural history of cervical human papillomavirus infections and cervical cancer: gaps in knowledge and future horizons. Obstet Gynecol Clin North Am. 2013;40(2):165-76.
32. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol. 2010;117(2 Suppl):S5-10.
33. Castle PE, Jeronimo J, Schiffman M, Herrero R, Rodriguez AC, Bratti MC, et al. Age-related changes of the cervix influence human papillomavirus type distribution. Cancer Res. 2006;66(2):1218-24.

34. Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA. 2001;285(11):1500-5.
35. Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med. 2007;357(16):1579-88.
36. Cox JT, Castle PE, Behrens CM, Sharma A, Wright TC, Jr., Cuzick J, et al. Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study. Am J Obstet Gynecol. 2013;208(3):184 e1- e11.
37. Ronco G, Dillner J, Elfstrom KM, Tunesi S, Snijders PJ, Arbyn M, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2013.
38. Wheeler CM, Hunt WC, Cuzick J, Langsfeld E, Robertson M, Castle PE. The Influence of type-specific human papillomavirus infections on the detection of cervical precancer and cancer: A population-based study of opportunistic cervical screening in the united states. Int J Cancer. 2013.
39. Gravitt PE. Evidence and impact of human papillomavirus latency. The open virology journal. 2012;6:198-203.

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Education

9/2006 - Present	Candidate for Doctor of Philosophy in Epidemiology Johns Hopkins Bloomberg School of Public Health Concentration: Infectious Diseases
8/2000-5/2002	Master of Public Health University of California Berkeley Concentration: Epidemiology/Biostatistics Specialty Area Certificate: Maternal and Child Health
9/1996-5/2000	Bachelor of Arts Johns Hopkins University Major: English

Professional Experience

8/2013 – Present	Research Project Manager, Health Services Research and Implementation Science, Department of Research and Evaluation, Kaiser Permanente Southern California (KPSC) Manage daily research operations for 6+ Care Improvement Research Team (CIRT) research projects. Plan and maintain all activities within the five-year, ~$10 million CIRT research portfolio. Contribute to research study design, analysis, quality assurance, and interpretation of results. Write and present poster and oral presentations, written reports and manuscripts.

Lead development of protocols for study operations including data validation using chart abstraction form electronic health record.
Supervise, train, and manage three research associates and multiple physician residents.
Interface with analytical programmers and biostatisticians to clarify analytic needs.
Plan, develop, and maintain staffing effort to ensure success of CIRT projects.
Oversee, prepare, and maintain all IRB-related submissions, continuing review reports, modifications, and study closure reports for all CIRT projects.
Coordinate and manage communication with internal and external partners including clinical and operational leaders, research scientists, post-doctoral fellows, and analysts within KPSC and across three KP regions and outside health care systems.
Uphold compliance and maintain confidentiality of protected health information (PHI) by developing and maintaining protocols and training staff to ensure utmost care with member PHI.

8/2010 – 10/2010 Research Specialist (Contract), National Tuberculosis Controllers Association

Developed and piloted standardized assessment tool to systematically characterize tuberculosis (TB) programs, staffing, policies, and funding in states and large cities throughout the United States.
Managed timely implementation of a national survey using a web-based delivery method.
Performed data collection, cleaning, and analysis of the resulting dataset.
Rapidly interpreted results and produced a summary report for distribution and oral presentation at the CDC Division of TB Elimination consultation on the impact of the Patient Protection & Affordable Care Act on U.S. public health programs responsible for TB control.

9/2007 – 8/2009 Graduate Research Assistant, Johns Hopkins Bloomberg School of Public Health

Collaborated with Principal Investigator, Dr. Patti Gravitt, on design, development, and pre-testing of baseline and follow-up survey instruments to assess hormonal, behavioral, epidemiological, and clinical risk factors for human papillomavirus (HPV) infection in women during perimenopause.
Conducted in-depth, baseline telephone interviews with study participants including sensitive topics.

Performed data entry and ensured data accuracy of completed questionnaires and clinical case report forms.

10/2006 – 5/2007 Public Health Applications for Student Experience Intern, Maryland Department of Mental Health and Hygiene

Abstracted primary data from medical charts at three, high-burden county public health clinics, created Access database, and performed data entry and cleaning for ~600 active tuberculosis (TB) cases with diabetes.
Analyzed and presented results of a statewide retrospective study examining the prevalence of and treatment outcomes associated with active TB cases with co-morbid diabetes mellitus.
Drafted report and manuscript to share findings.

9/2004 – 6/2006 Epidemiologist and Program Manager (Analyst IV), Francis J. Curry National Tuberculosis Center, University of California San Francisco

Conducted operational research to develop, implement, and evaluate best-practice models for regional capacity-building across four U.S. states with low TB incidence (CDC Division of Tuberculosis Elimination Task Order 6).
Designed standardized tools and protocols to effectively collect, manage, and apply regional epidemiologic and genotypic data to optimize local TB control activities in Idaho, Montana, Utah, and Wyoming.
Initiated, disseminated, and analyzed two mycobacteriology laboratory services assessments to guide educational interventions.
Constructed regional and state epidemiologic profiles to support advocacy efforts to control and eliminate TB.
Created and managed budgets and timelines for multiple projects within the cooperative agreement.
Managed program assistants, interviewers, and data entry staff to ensure efficient implementation and quality assurance activities as part of a multi-center, cross-sectional study to identify missed opportunities for preventing TB transmission in foreign-born populations.

4/2003 – 9/2004 Senior Epidemiologist, Infant Botulism Treatment & Prevention Program, California Department of Health Services

Collected, evaluated, and prepared reports of surveillance and laboratory data on infant botulism cases in California and the United States.
Conducted cost-savings analyses of experimental and post-

licensure distribution of an orphan drug treatment for infant botulism (BabyBIG®).
Evaluated and presented clinical data for safety and efficacy of the orphan drug for periodic reporting to the United States Food and Drug Administration.
Trained and supervised research assistants and undergraduate student workers including proper data collection and quality control procedures.
Provided one-on-one education and support for affected families via telephone.
Guided design and implementation of program website (www.infantbotulism.org) and monitored online parent support forum.

Professional Activities

3/2014 – Present	Lean Six Sigma Black Belt certification, ProgressivEdge Demonstrate mastery of process improvement techniques by applying various tools to achieve >$100k annualized cost savings by eliminating waste and reducing variation in data collection and validation processes used for multiple health services research projects
2010 – Present	Student Member, Society for Epidemiologic Research
9/2010 – 8/2011	Coordinator, Sexually Transmitted Infections Journal Club, Johns Hopkins Bloomberg School of Public Health
9/2008 – 5/2009	Coordinator, Infectious Disease Epidemiology Journal Club, Johns Hopkins Bloomberg School of Public Health
9/2004 - 6/2006	Planning Committee Member, California Tuberculosis Epidemiologists Network

Honors and Awards

9/2009 – 8/2011	Pre-doctoral Fellow, National Institutes of Health Sexually Transmitted Infections Training Grant (T32-AI050056)
2000	Public Health Alumni Association Scholarship, University of California Berkeley

Publications

Gould MK, Franco AS, Liu I-L, **Tang T**, Slezak J. Identification of patients with hospital-acquired acute respiratory failure using structured data from electronic health records. Am J Respir Crit Care Med (in press).

Dooley KE, **Tang T**, Golub JE, Dorman SE, and Cronin W. Impact of diabetes mellitus on treatment outcomes of patients with active tuberculosis. Am J Trop Med Hyg. 2009 Apr;80(4):634-9.

Barash JR, **Tang TWH**, Arnon SS. First case of infant botulism caused by *Clostridium baratii* type F in California. J Clin Microbiol 2005;43:4280-2.

Tandri H, Griffith L, **Tang T**, Nasir K, Zardkoohi O, Reddy CV, Capps M, Calkins H, Donahue JK. Clinical course and long-term follow-up of patients receiving implantable cardioverter-defibrillators. Heart Rhythm. 2006 Jul;3(7):762-8. Epub 2006 Mar 27.

Teaching

2007-2010 Graduate Teaching Assistant, Johns Hopkins Bloomberg School of Public Health

Fostered understanding of epidemiologic concepts and theory and guided practical application of epidemiologic methods in laboratory sessions of 20-30 graduate students
Attended lectures and teaching assistant meetings, held weekly office hours, graded homework assignments and written exams

Courses:
- Epidemiologic Methods for Planning and Evaluating Health Services, Dr. Carlos Castillo (Summer 2008, Summer 2010)
- Principles of Epidemiology, Dr. William Moss (Fall 2008)
- Principles of Epidemiology, Dr. Lechaim Naggan (Summer 2008)
- Epidemiologic Basis for Tuberculosis Control, Drs. Jonathan Golub, Richard Chaisson, and George Comstock (Summer 2007)

Presentations

Hahn EE; **Tang T**; Lee JS; Munoz-Plaza C; Shen E; Rowley B; Adesina JO; Maeda JL; Mosen DM; Ruckdeshel JC; Gould MK: Use of imaging for staging of early stage breast cancer within two integrated health care systems. American Society of Clinical Oncology Quality Symposium: Boston, MA; October 17-18, 2014.

Hahn EE; **Tang T**; Lee JS; Munoz-Plaza C; Adesina JO; Maeda JL; Mosen DM; Gould MK: Measuring the American Society of Clinical Oncology Choosing Wisely "Top Five" list within an integrated health care system: Provider level variation in biomarker utilization. Biennial Cancer Survivorship Research Conference: Advancing Survivorship Through Multi-Level Collaborations. Atlanta, GA; June 18-20, 2014.

Hahn EE; **Tang T**; Lee JS; Munoz-Plaza C; Adesina JO; Maeda JL; Mosen DM; Gould MK: Measuring the American Society of Clinical Oncology Choosing Wisely "Top Five" list: The importance of clinical indication in cancer quality measurement. Academy Health Annual Research Meeting. San Diego, CA; June 8-10, 2014.

Gould MK, Franco AS, Slezak JM, Liu ILA, **Tang T**. Identification of patients with hospital-acquired acute respiratory failure using structured data from electronic

health records. American Thoracic Society International Conference, San Diego, CA, May 2014.

Sharp AL; Nguyen HQ; Hahn EE; **Tang T**; Mittman BS; Jacobsen SJ; Kanter MH; Gould MK: The Just Do It Playbook: A Practical Implementation Framework. HMO Research Network Annual Meeting: Embedded Research to Improve Health. Phoenix, AZ. March 31-April 3, 2014.

Gould MK; Nguyen HQ; Sharp AL; Hahn EE; **Tang T**; Mittman BS; Jacobsen SJ; Kanter MH: Research-Operations Partnerships to Improve the Quality and Affordability of Care. HMO Research Network Annual Meeting: Embedded Research to Improve Health. Phoenix, AZ. March 31-April 3, 2014.

Tang T. Impact of diabetes on treatment outcomes among Maryland tuberculosis cases, 2004–2005. Public Health Applications for Student Experience (PHASE) Symposium, Baltimore, MD, May 2007.

Abernethy NF, Pascopella LP, Reves R, **Tang T**. Tuberculosis laboratory services assessment in a multi-state low-incidence region. International Union Against Tuberculosis and Lung Disease, North American Region, Vancouver, British Columbia, February 2007.

Tang T. Current tuberculosis epidemiology and program priorities in the U.S.-Mexico border region. Tuberculosis Update, Tijuana, Mexico, November 2005.

Tang T. Regional tuberculosis laboratory services assessment. Rocky Mountain TB Controllers Meeting, Boise, ID, August 2005.